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STUDIES ON THE PHYSIOLOGY OF RICE

X. EFFECTS OF POTASSIUM DEFICIENCY ON GROWTH AND NITROGEN METABOLISM

By S. M. Sircar and S. C. Datta

(Received for publication on September 16, 1954)

(With 12 Text-Figures)

SINCE crops differ markedly in their response to potassium, it is worthwhile to investigate the effects of K-deficiency on rice plant. Its importance lies in the fact that, for rice, ammonium sulphate is used as fertiliser. In connection with ammonium salts, one great theoretical consideration is the rate at which the ammonium ion is metabolised to organic nitrogen as otherwise its accumulation will lead to toxicity. It has been suggested that potassium plays an important role in the synthesis of amino acids and proteins. Plants growing in solution high in ammonium salts and low in potassium fail to form ear or die prematurely on account of ammonium accumulation [Richards, 1938].

In view of these considerations, it is desirable to determine the effects of potassium deficiency on growth, nitrogen metabolism and grain yield of rice plant. This article embodies the results obtained from growing rice in sand culture, using different levels of potassium in conjunction with nitrate and ammonium ions.

Experimental

A pure line of winter paddy, *Bhasamanik*, was grown in sand-culture in the summer of 1952. The technique of sand-culture adopted was the same as in the previous work [Sircar and Sen, 1941]. In all 200 glazed pots were used in this experiment.

Rice seeds of uniform size and colour were selected and sterilised with 0·2 per cent formalin for six hours. Nine seeds were sown on June 23 ; germination began on June 25 and was practically complete within five days. When the seedlings formed first two leaves, the plants were thinned to three per pot with uniformity in size and space.

The manurial scheme employed in this work was that of [Gregory and Richard, 1929] for the cultivation of barley. This has also been tried successfully in wheat [Sircar, 1939] and rice [Sircar and Sen, 1941] in our laboratory. It has been suggested that both ammonium and nitrate ions are indispensable for rice [Dastur and Malkani, 1933]. [Mullison and Mullison, 1942] noted that in a study of potash

nutrition sodium should be excluded from the nutrient in order to obtain a graphic picture of the role of potassium in plant metabolism. Hence sodium nitrate was retained in a small number of cases, but ammonium nitrate formed the main plank of the experiment.

The weights of salts added per pot were :

Sodium nitrate ($NaNO_3$)	3·00 gm.
Or	
Ammonium nitrate (NH_4NO_3)	3·00 gm.
Sodium hydrogen phosphate (Na_2HPO_4, $12H_2O$)	2·52 gm.
Potassium sulphate (K_2SO_4)	1·85 gm.
Magnesium sulphate ($MgSO_4$, $7H_2O$)	1·27 gm.
Calcium chloride ($CaCl_2$, $6H_2O$)	0·37 gm.
Ferric chloride ($FeCl_3$, $6H_2O$)	0·10 gm.
Manganese sulphate ($Mn\ SO_4$)	0·10 gm.

For potassium deficiency 1/3rd, 1/27th and 1/81st of the standard amount were used. The four sets were designated as full manure (1·00K), 1/3rd (0·33K), 1/27th (0·03K) and 1/81st (0·01K). There were 40 pots (20 for control and 20 for deficiency) in the nitrate series and 160 (40 for control and 40 each for the three deficiencies) in the ammonium series. The nutrients were applied to the young plants in dilute solutions in three equal doses at fortnight intervals. The *p*H of the nutrient solution was adjusted between 6·7 to 7·0. The first dose of nutrient solution was applied after the plants were thinned down on July 8 ; the second dose on July 22, and the third on August 5.

In each set half of the pots were used for growth analyses, whilst the other half for leaf sampling. The leaves on the main axis were marked with Indian ink and were sampled for nitrogen analyses. When the ears in each plant ripened, the grains were harvested and their air dried weights recorded.

Analytical Methods

Six to ten leaves, just at the time of their complete maturity, were sampled from the main shoot of leaf numbers 13, 14, 15, 16, 19 and 21 and were bisected longitudinally. One half was used for the determination of total nitrogen by the micro-Kjeldahl apparatus modified by Parnas and Wagner as described by Pregl [1930]. The other half was ground in a glass mortar to paste and the extract filtered through paper pulp in a Buchner funnel. Protein was removed by adding 50 per cent trichloro-acetic acid to the leaf extract and from the filtrate crystalloidal nitrogen was determined as before by the same micro-Kjeldahl technique.

Free ammonia was estimated by distilling in vacuo the aqueous extract with a thick cream of magnesium oxide at room temperature. Total amino nitrogen was determined by the Van Slyke micro amino-apparatus, using a reaction time of 55 minutes [Loomis and Shull, 1937]. Amide nitrogen was determined by hydrolysing protein-free extract with 0·25N sulphuric acid for four hours and ammonia evolved at 40°C was estimated by Wolff's method [1928]. Amino acid nitrogen was calculated from the difference between the total amino nitrogen and amide nitrogen.

Nitrate and nitrite nitrogen was estimated with slight modification. The method is based on the assumption that nitrates and nitrites in the presence of hot alkali and Devarda's alloy emit pure ammonia.

Results

The results were as follows:

Deficiency symptoms

The symptoms induced by potassium deficiency included slender plants of which some withered away in course of a few weeks. The control plants were bushy in habit and showed vigorous growth. The K-starved plants exhibited reduction in stem length and increased succulence.

The stems were slender and displayed the phenomenon of "coppering", i.e. development of minute brown streaks on their surface.

The leaves were dull green to yellow in colour. Some of them showed the formation of red patches on the edge, possibly due to the development of anthocyanin. The older leaves were first affected by the impact of potassium deficiency. They were rolled up and "scorched" which commenced at the tip and proceeded towards the base (Table I). The leaves at emergence were green, but became chlorotic when unfolded.

Table I

Remarks concerning leaves analysed

Treatment	Remarks
Leaf No. 13	
$NaNO_3$ with 1K	Tips very slightly dry ; pale green to yellowish green in colour
$NaNO_3$ with 0·01K	Tips having a length of 2 cm. were dry ; yellowish in colour
NH_4NO_3 with 1K	Tips not dry ; green in colour

TABLE I—(contd.)

Remarks concerning leaves analysed

Treatment	Remarks
	Leaf No. 13
NH_4NO_3 with 0·33K	Tips slightly dry ; pale green in colour
NH_4NO_3 with 0·03K	Tips having a length of 1·3 cm. were dry ; yellowish green in colour ; slightly red towards the tip
NH_4NO_3 with 0·01K	Tips having a length of 1·7 cm. were dry ; yellowish green in colour ; much redder than 0·03K with bronze coloured spots in the leaf surface
	Leaf No. 14
$NaNO_3$ with 1K	Tips slightly dry ; pale green to green in colour
$NaNO_3$ with 0·01K	Tips having a length of 2·2 cm. were dry ; yellowish green in colour
NH_4NO_3 with 1K	Tips not dry ; yellowish green to green in colour
NH_4NO_3 with 0·33 K	Tips slightly dry ; yellowish green in colour ; becoming rolled towards the upper end
NH_4NO_3 with 0·03K	Tips having a length of 2·4 cm. dry ; pale green to yellowish green in colour ; reddened towards the edge ; becoming rolled towards the upper end
NH_4NO_3 with 0·01K	Tips very much reddened and slightly dry ; green in colour with bronze coloured spots in the leaf surface
	Leaf No. 15
$NaNO_3$ with 1K	Tips slightly dry ; green in colour
$NaNO_3$ with 0·01K	Tips much reddened than drying ; yellowish green in colour
NH_4NO_3 with 1K	Tips not dry ; yellowish green to green in colour
NH_4NO_3 with 0·33K	Tips slightly dry ; yellowish green in colour ; becoming rolled towards the distal end
NH_4NO_3 with 0·03K	Tips having a length 2·6 cm. dead ; pale green to yellowish green in colour
NH_4NO_3 with 0·01K	Leaves half dead ; pale green in colour ; studded with bronze coloured spots

TABLE I—(contd.)

Remarks concerning leaves analysed

Treatment	Remarks
	Leaf No. 16
$NaNO_3$ with 1K	Tips slightly dry ; green in colour
$NaNO_3$ with 0·01 K	Tips rolled up ; yellowish green in colour
NH_4NO_3 with 1K	Tips not dry ; yellowish green to green in colour
NH_4NO_3 with 0·33K	Tips dried up and rolled ; yellowish green in colour ; studded with minute brown spots on the lamina
NH_4NO_3 with 0·03K	Tips much drier than 0·33K ; yellowish green in colour ; studded with minute brown spots on the leaf blade
NH_4NO_3 with 0·01K	Tips much reddened than drying ; studded with minute brown spots
	Leaf No. 19
$NaNO_3$ with 1K	Tips slightly dry ; green in colour
$NaNO_3$ with 0·01K	Tips slightly dry ; yellowish green in colour ; studded with brown spots
NH_4NO_3 with 1K	Tips not dry ; green in colour
NH_4NO_3 with 0·33K	Tips slightly dry and insect bitten ; green in colour
NH_4NO_3 with 0·03K	Tips much drier than 0·33K ; yellowish green in colour
NH_4NO_3 with 0·01K	Tips not expanded and very much dry ; yellowish green in colour
	Leaf No. 21
$NaNO_3$ with 1K	Tips not dry ; green in colour
$NaHO_3$ with 0·01K	Tips slightly dry and rolled up ; yellowish green in colour
NH_4NO_3 with 1K	Tips not dry ; green in colour
NH_4NO_3 with 0·33K	Leaves one-third dry ; green in colour
NH_4NO_3 with 0·03K	Leaves not expanded and half dry ; yellowish green in colour
NH_4NO_3 with 0·01K	Leaves reduced in size, one-third dry and insect bitten ; yellowish green in colour

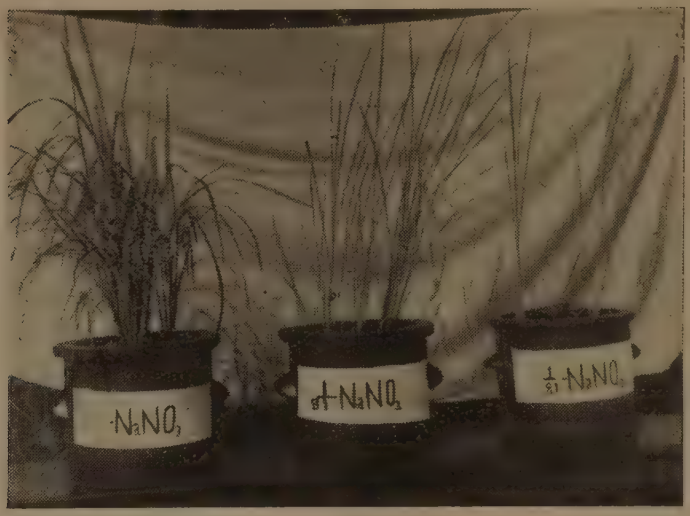

FIG. 1. Rice plants var. *Bhasamanik* grown under potassium deficiency

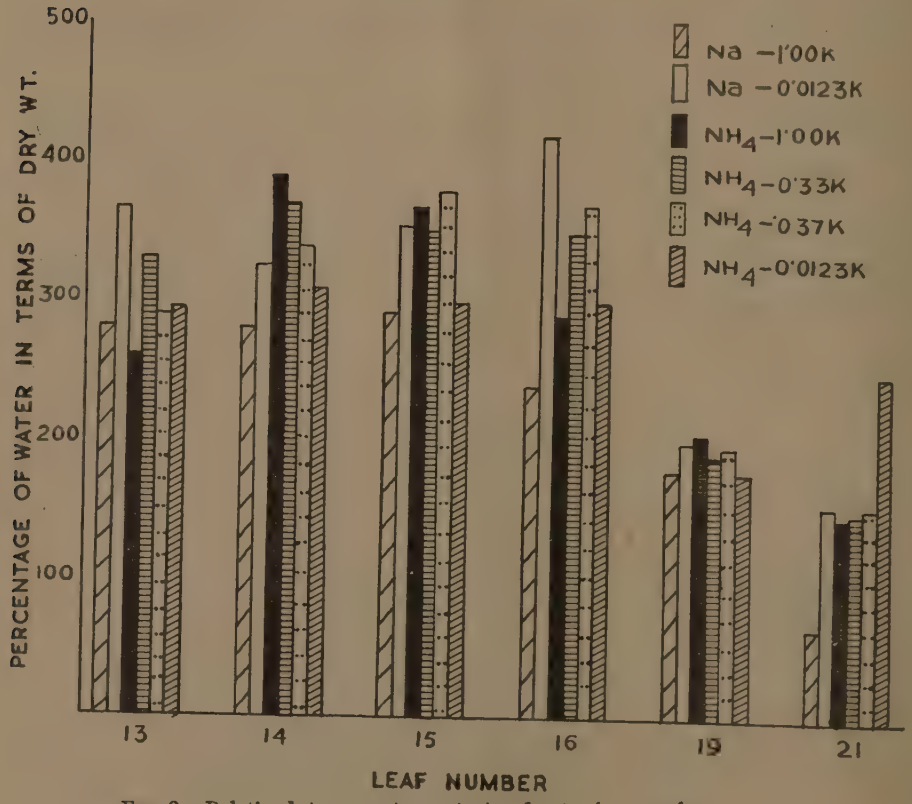

FIG. 2. Relation between water content and potassium supply

The roots were long with a number of lateral branches and assumed a yellowish appearance.

An interesting feature was the emergence of ears in the deficiency levels and a few individuals failed to produce reproductive organs. In addition to this, there was immature development of small seeds.

Water content

The water content increased progressively with decreasing levels of potassium. This was true in all the leaves of the plants receiving sodium nitrate, but not in the ammonium nitrate series. In the latter, marked fluctuations in the water content at different levels occurred except in leaf No. 21 where a progressive increase in water content was noticed (Fig. 2).

Growth analysis

Measurement of height. The height of the plants was measured from the fourth week after sowing (Table II). During the first three weeks all the plants were indistinguishable, differences becoming prominent from the fourth week onwards. From the inception of the fifth week, differences in the general height of the plants in the sodium nitrate series were noticeable and this was seen in the ammonium nitrate series after the eighth week. Thus increased potassium deficiency led to progressive reduction in stem elongation of the rice plant.

TABLE II

Height in cm.

Weeks after sowing	$NO_3$1K	$NO_3$1/81K	NH_4 1K	$NH_4$1/3K	$NH_4$1/27K	$NH_4$1/81K
IV	32·99	32·30	28·75	24·40	23·16	26·41
V	38·00	36·91	36·05	35·63	28·27	31·21
VI	43·54	42·29	40·70	39·59	33·38	36·24
VII	46·00	45·53	44·42	41·90	38·48	41·29
VIII	50·67	48·67	50·60	49·56	45·21	43·46
IX	53·68	52·68	51·94	54·53	51·86	49·65
X	54·14	52·92	62·60	51·97	52·01	51·19
XI	59·92	56·40	67·37	62·24	56·38	52·63
XII	64·14	61·22	70·85	64·72	58·60	54·91
XIII	69·43	63·78	74·21	67·64	61·89	57·75
XIV	71·82	66·64	77·29	69·00	62·76	59·78
XV	75·38	68·00	79·32	71·11	66·37	61·89

The growth data were analysed statistically by the technique of the analysis of variance. When the two levels of potassium were applied along with $NaNO_3$ the heights did not show differences up to the tenth week except in the seventh and eighth weeks. From the eleventh week, the differences were significant, and became more and more pronounced as the plants grew older. With NH_4NO_3 except in the eleventh week, the differences were significant at all levels of potassium supply. Thus, the effect of potassium on the general height of the plants was more conspicuous in the ammonium nitrate series than the sodium nitrate one.

Counting of tillers. Tiller counting was also made from the fourth week onwards and the average values are shown in Fig. 3. The difference in the number of tillers produced per plant became prominent in the sodium nitrate series from the seventh week and in the ammonium nitrate series from the sixth week.

In the sodium nitrate series the differences in tillering were significant for 4, 5, 6, 11, 12, 13 and 15 weeks, while the ammonium nitrate series were, in general, significant and more marked for later periods.

Yield. The grain yield per pot showed a progressive fall with the different levels of deficiency (Table IV and Fig. 4). The decreased yield in the potassium-starved plants might be due to the poor setting of grains. A general remark concerning the grains is given in Table III. The results were significant at 5 per cent level in the nitrate series. But in the ammonium series the results were significant at 1 per cent level in four cases and at 5 per cent in one case.

TABLE III

Remarks concerning grains

Treatment	Remarks on grains
NO_3 with 1K	Only 5 per cent (0·397 gm. out of 7·813 gm.) did not fill
NO_3 with 1/81K	50 per cent (3·017 gm. out of 6·033 gm.) did not fill
NH_4 with 1K	Grains all filled
NH_4 with 1/3K	20 per cent (1·805 gm. out of 9·024 gm.) did not fill
NH_4 with 1/27K	30 per cent (2·413 gm out of 8·043 gm.) did not fill
NH_4 with 1/81K	40 per cent (2·596 gm. out of 6·489 gm.) did not fill

TABLE IV

Yield in gm.

Treatment	Yield per plant	Yield per pot
$NaNO_3$ with 1K	7·813	23·439
$NaNO_3$ with 0·01K	6·033	18·099
NH_4NO_3 with 1K	11·485	34·455
NH_4NO_3 with 0·33K	9·024	27·072
NH_4NO_3 with 0·03K	8·043	24·129
NH_4NO_3 with 0·01K	6·489	19·167

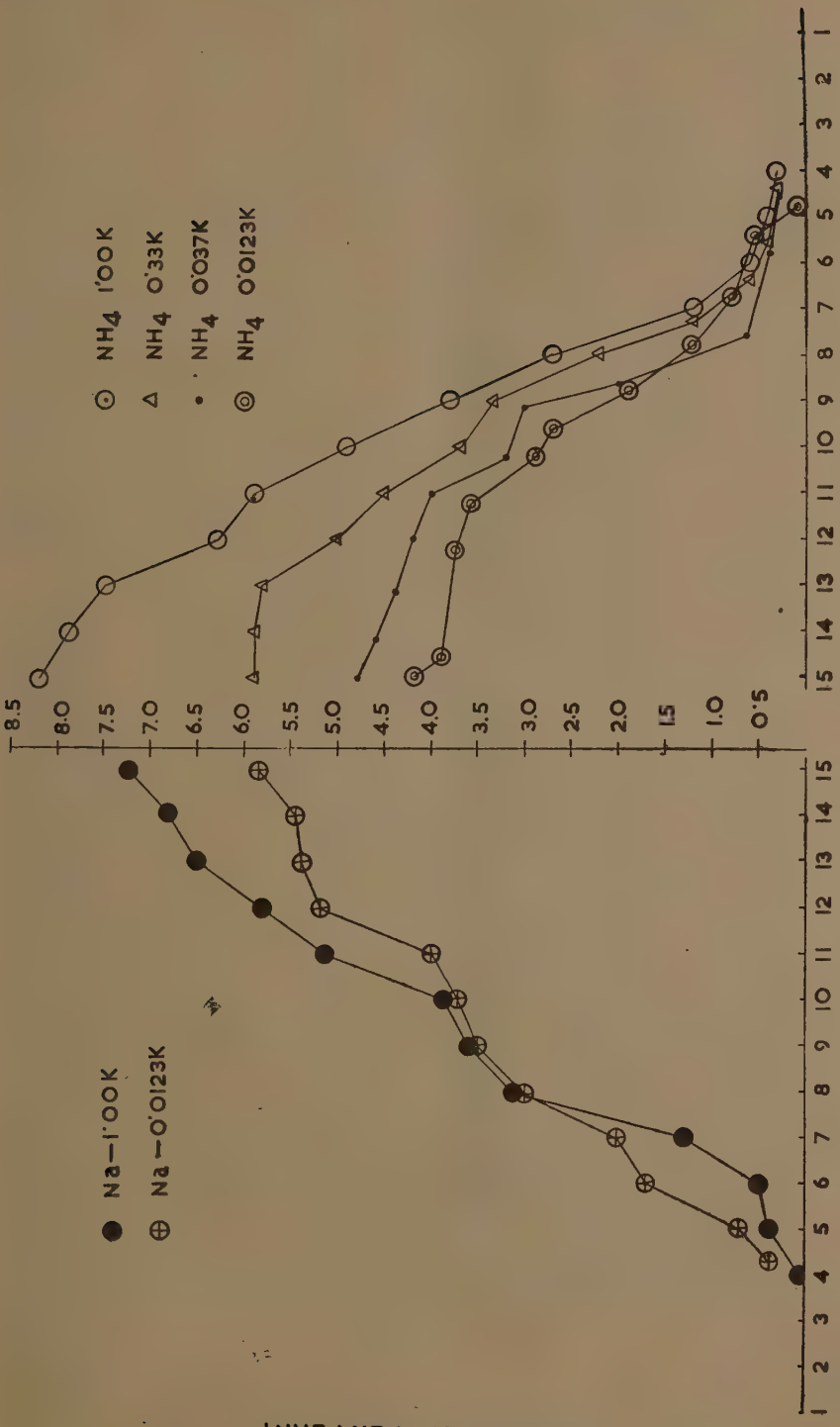

Fig. 3. Relation between tiller number and potassium supply

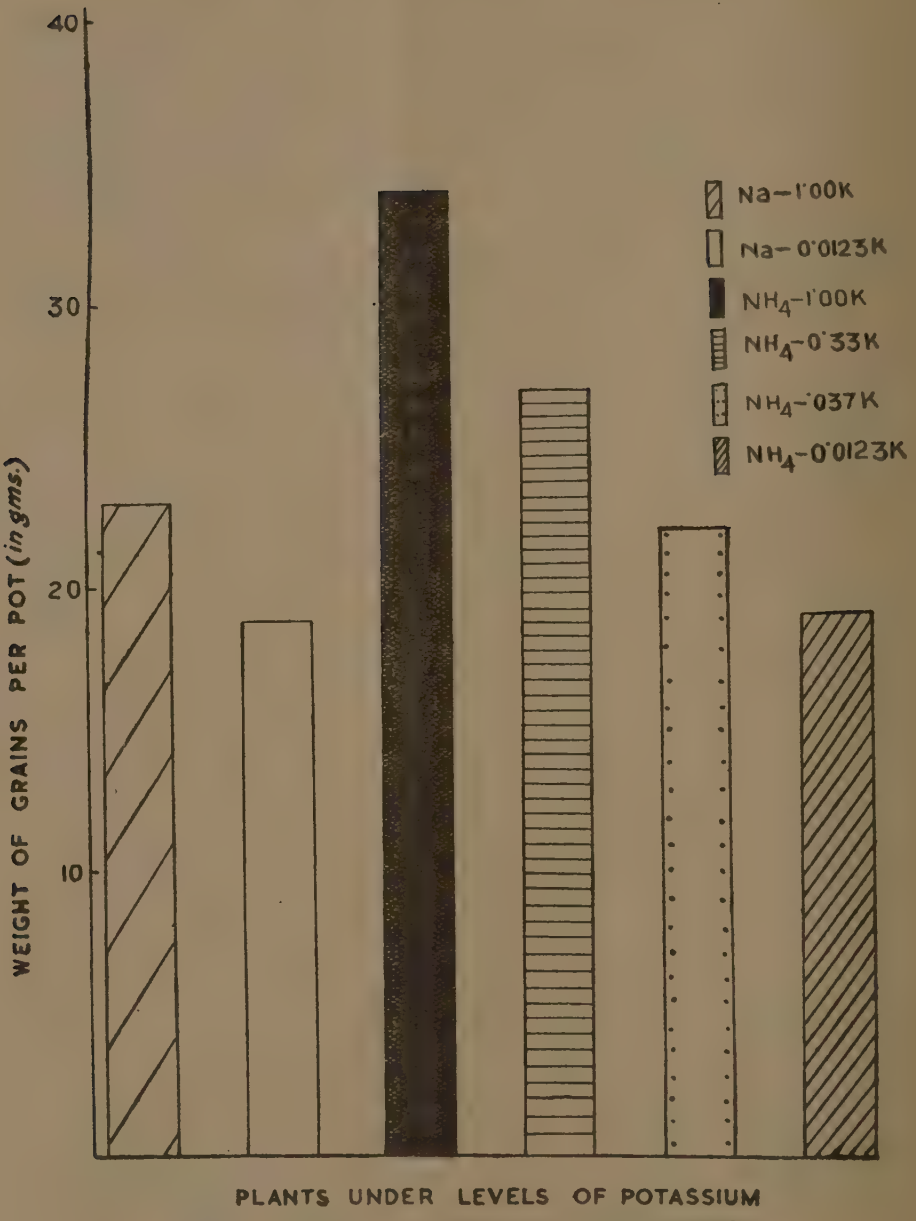

Fig. 4. Relation between yield per pot and potassium supply

Nitrogen Analyses

The results of nitrogen analyses of leaves are shown in Figs. 5, 6, 7, 8, 9, 10, 11, 12 and 13 as percentage of dry weight. As the analyses were made from a representative sample of leaves, it was worthwhile to note the differences between treatments without statistical analysis. From the general trend of behaviour in various leaf numbers the effect of potassium supply on nitrogen metabolism was remarkable.

The maximum content of total nitrogen was found in the full manure series and the level fell with decreasing potassium supply. These relations were observable in each of the six leaves analysed. After the 14th-leaf stage, the total nitrogen content decreased in the successive leaves of both the sodium nitrate and ammonium nitrate series and this fall was noticed in all the levels of potassium concentration. The 14th leaf of the ammonium series absorbed more nitrogen than that of the nitrate series. The uptake of nitrogen was, therefore, associated with the supply of potassium. In the 13th, 14th, 15th, 16th and 21st leaves of the full manure set with sodium nitrate, non-protein or crystalloidal nitrogen was high and the level fell with decreasing supply of potassium ; but the 19th leaf showed a deviation by having a high content of crystalloidal nitrogen. While in the ammonium nitrate series crystalloidal nitrogen was high in full manured set, it decreased under potassium deficiency. Minor fluctuations in crystalloidal nitrogen content were also noticed in different leaves. Protein nitrogen was high in both sodium and ammonium plants and dropped progressively with decreasing potassium supply. Under potassium deficiency there was a large accumulation of ammonia nitrogen. In the full manured set, a low ammonia nitrogen value was noted. Total amino nitrogen was highest in the leaves which exhibited potassium starvation. In sodium nitrate series, this nitrogen fraction showed fluctuating variation. But ammonium nitrate series exhibited a different picture ; here, total amino nitrogen was low in the full manured set, but increased progressively with decreasing supply of potassium. As separate estimations of ammonia nitrogen were made, the amide figures presented here exclude all the free ammonia that might be available. A large increase in amide nitrogen following the lowering of potassium supply was found in the leaves. The amino acid values increased in the ammonium nitrate series from the 13th to 15th leaf, but fell gradually in the 16th and 19th leaf stages. The content of nitrite and nitrate nitrogen increased with progressive deficiency of potassium.

After the ripe ears harvested, they were subjected to the usual process of air-drying and then pounded in an iron mortar. The powdered material was used for the analyses of different fractions and the data have been presented in Table V. The total nitrogen and protein nitrogen contents were highest in the full manure series and decreased with progressive deficiency of potassium. On the other hand, total amino nitrogen, amide nitrogen and amino acid nitrogen increased progressively in the potassium starved ears. Nitrite and nitrate and ammonia nitrogen showed large accumulation in the deficiency levels. Lastly, non-protein nitrogen decreased with the decreasing levels of potassium.

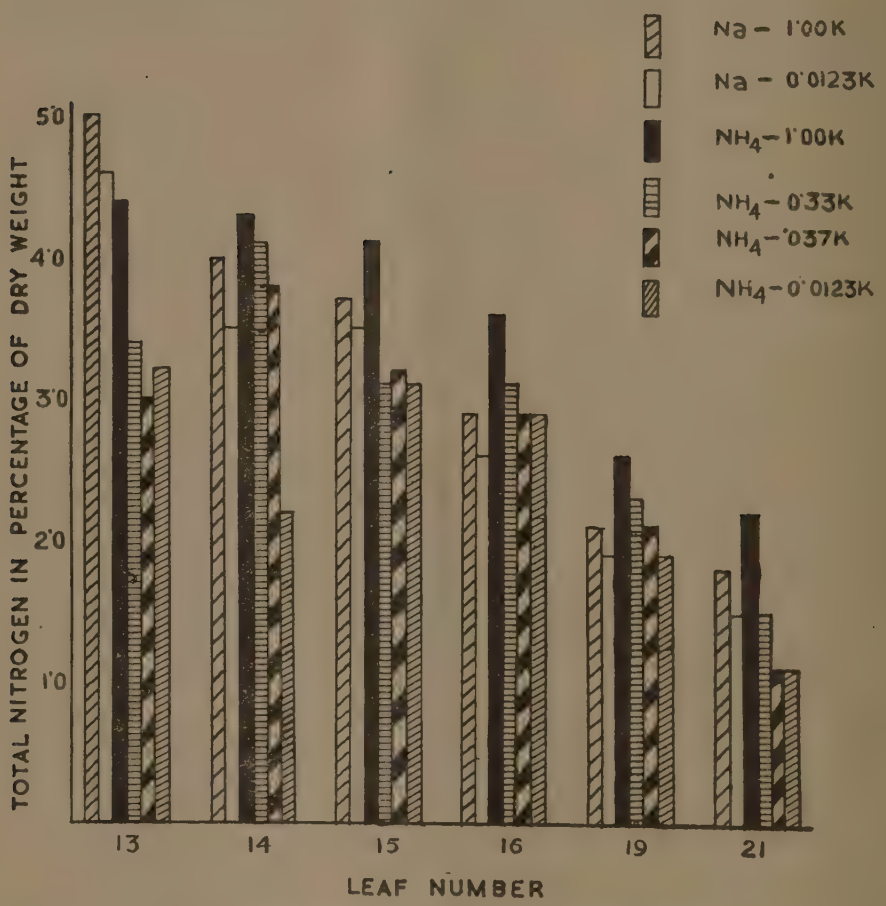

FIG. 5. Relation between total nitrogen and potassium supply

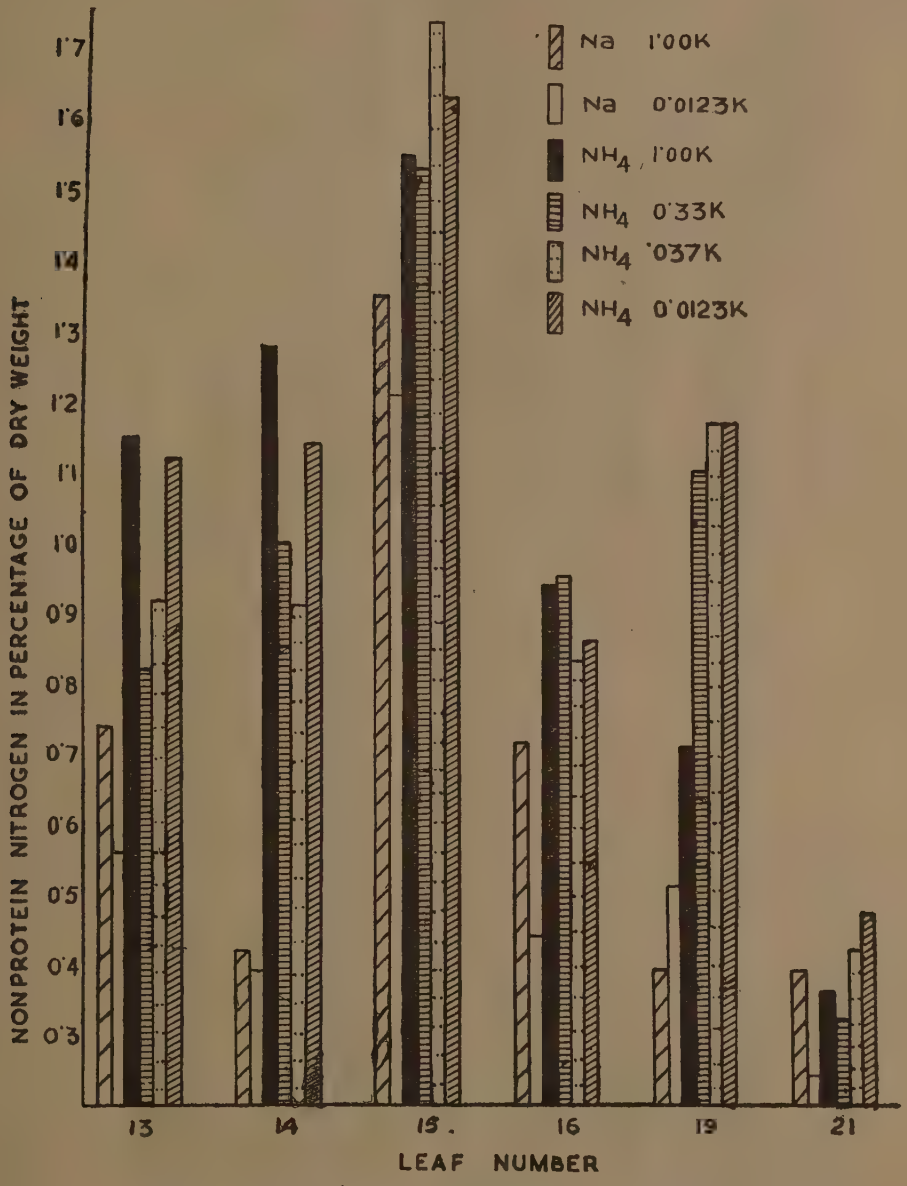

Fig. 6. Relation between non-protein nitrogen and potassium supply

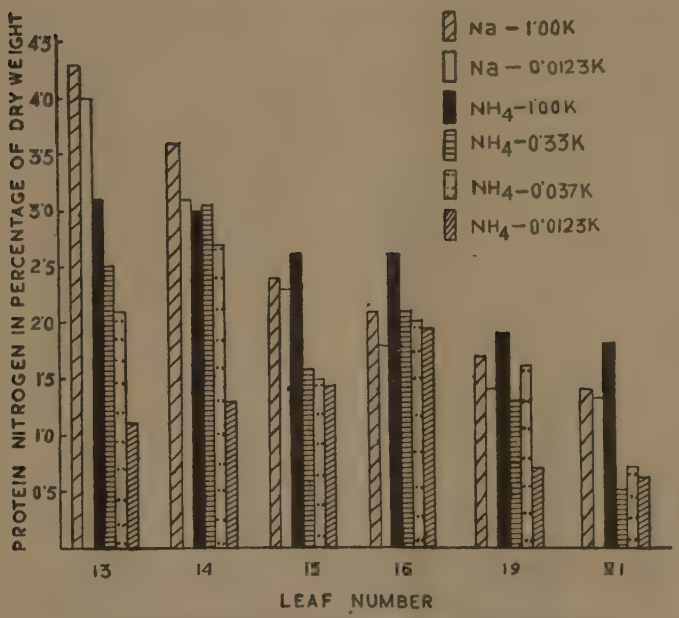

FIG. 7. Relation between protein nitrogen and potassium supply

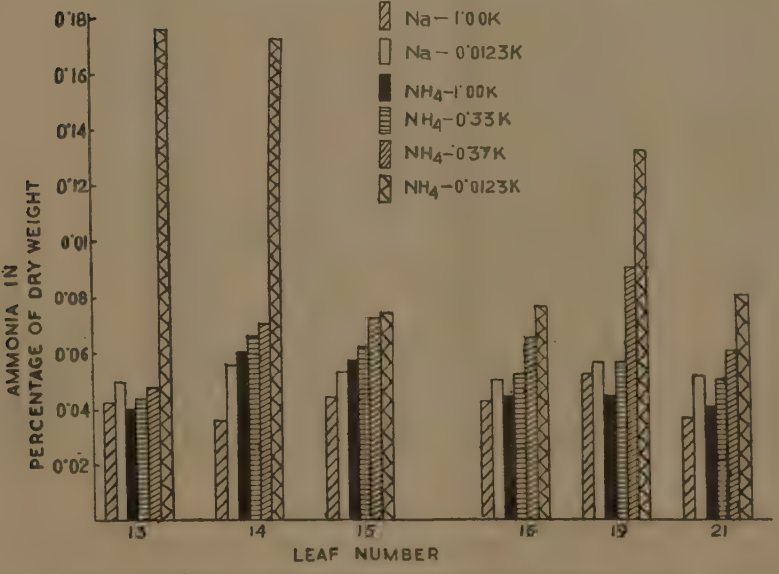

FIG. 8. Relation between ammonia nitrogen and potassium supply

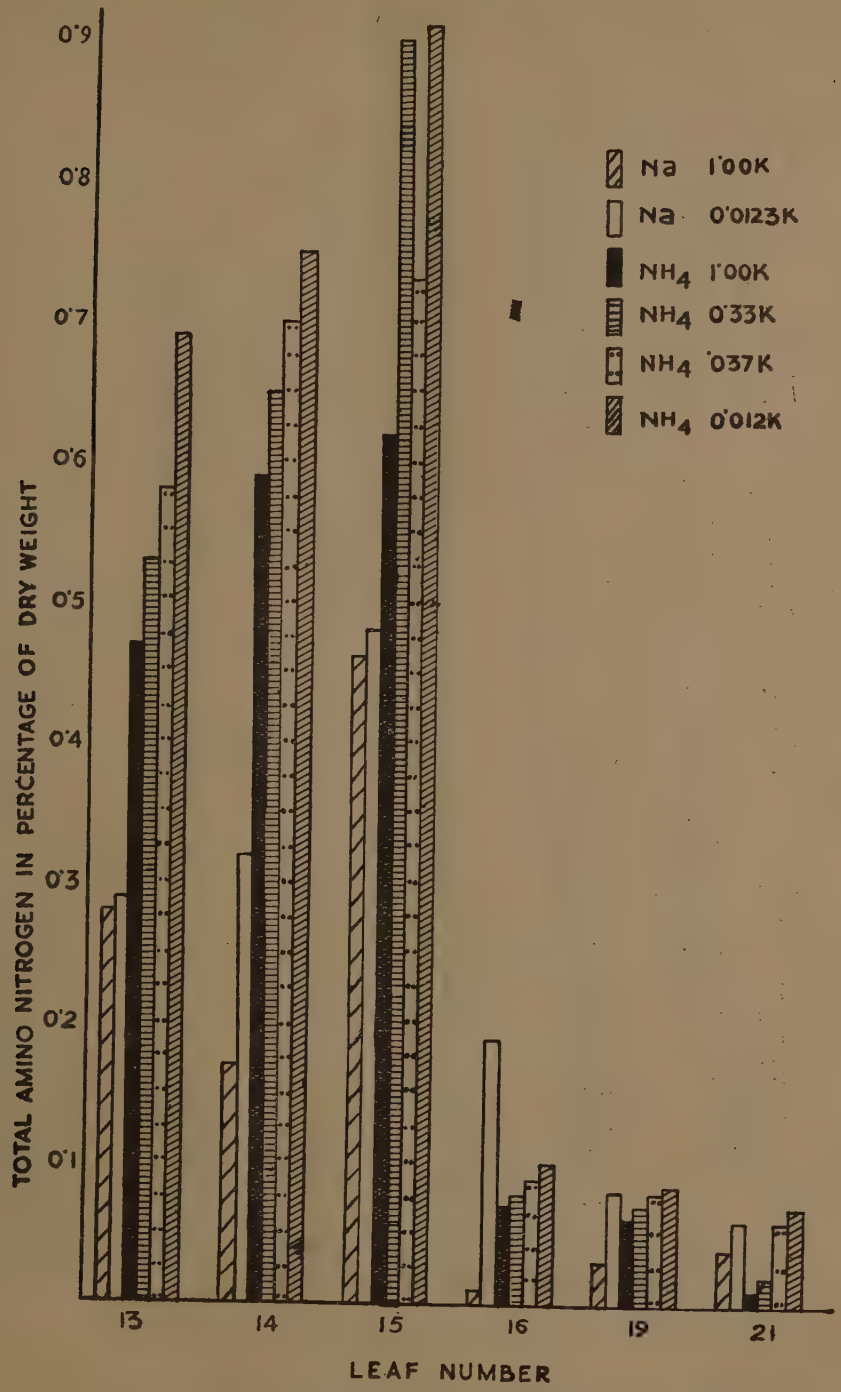

FIG. 9. Relation between total amino nitrogen and potassium supply

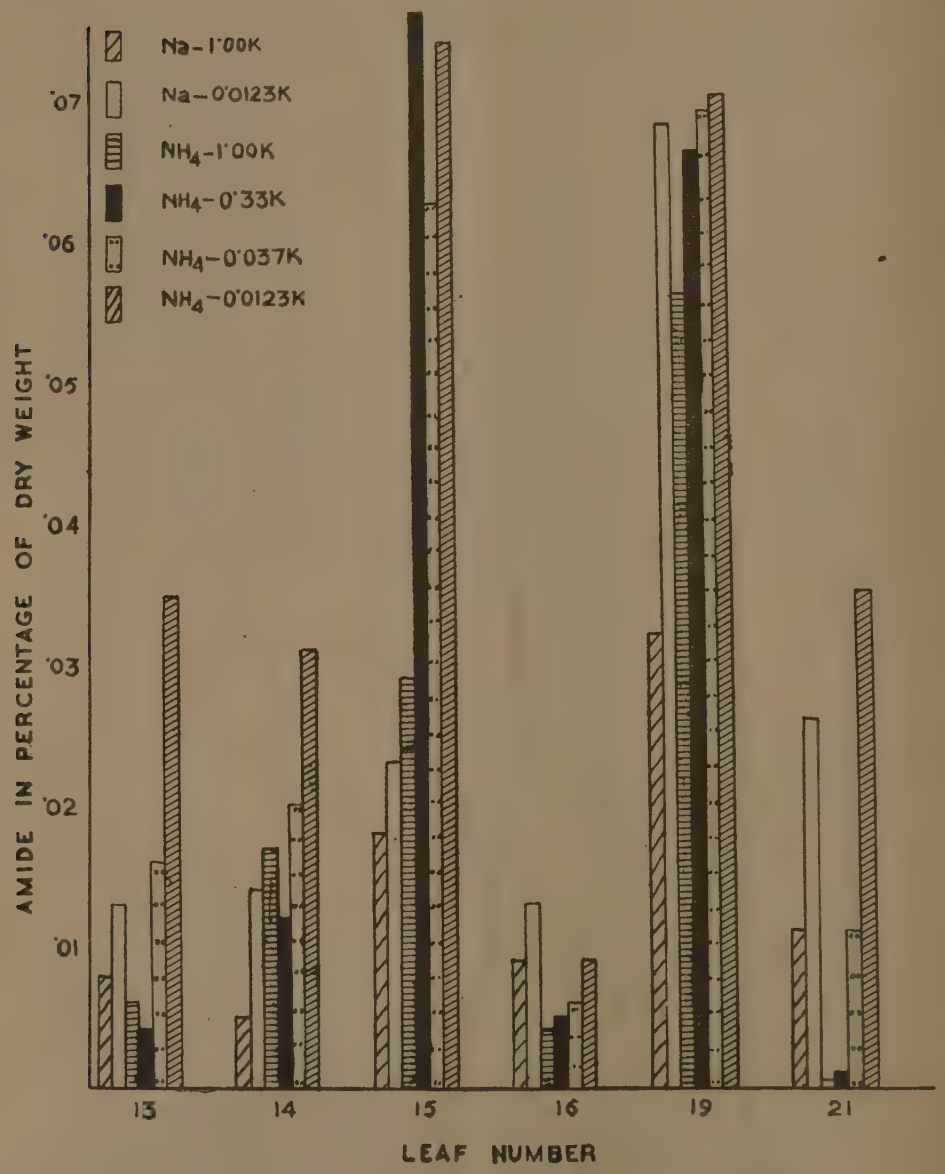

FIG. 10. Relation between amide nitrogen and potassium supply

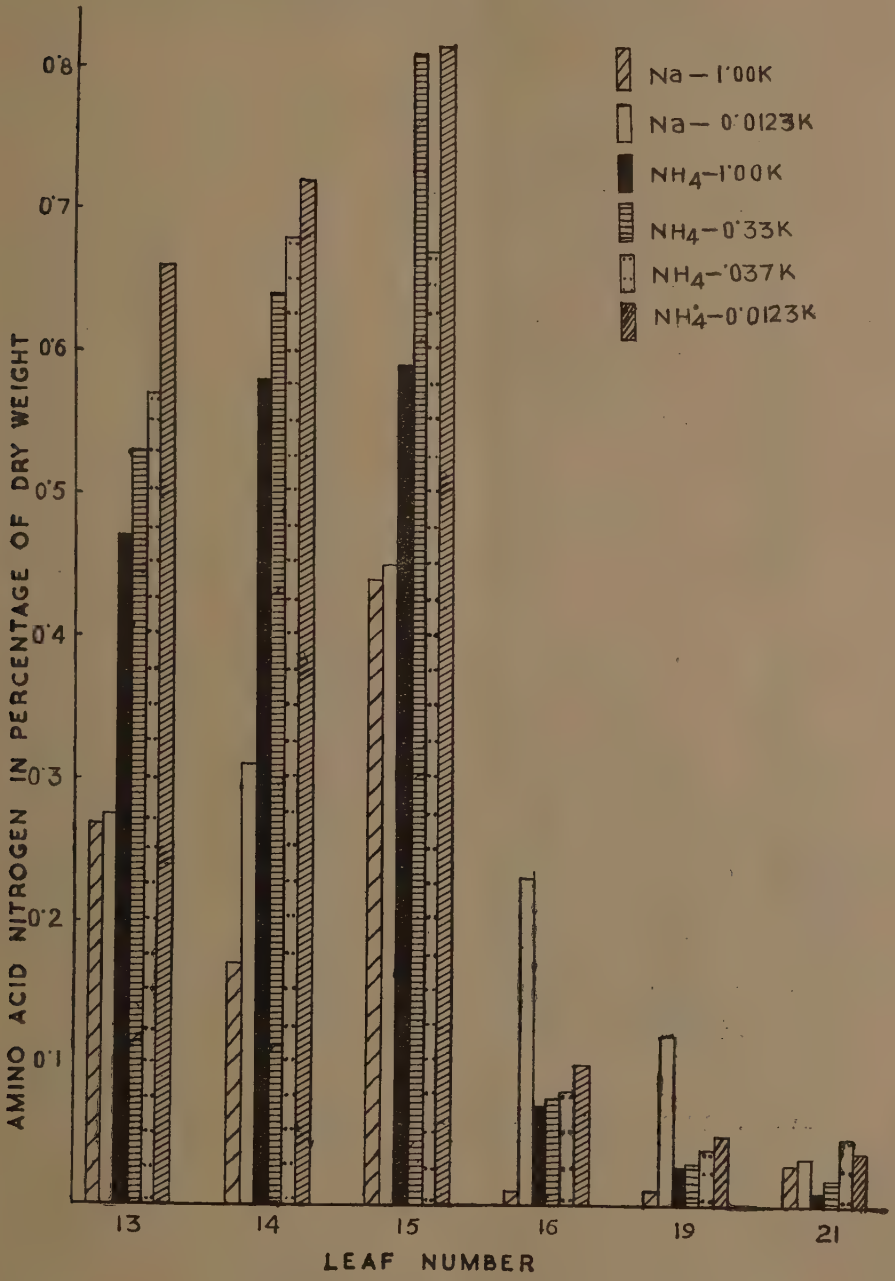

Fig. 11. Relation between amino acid nitrogen and potassium supply

55 AR/56

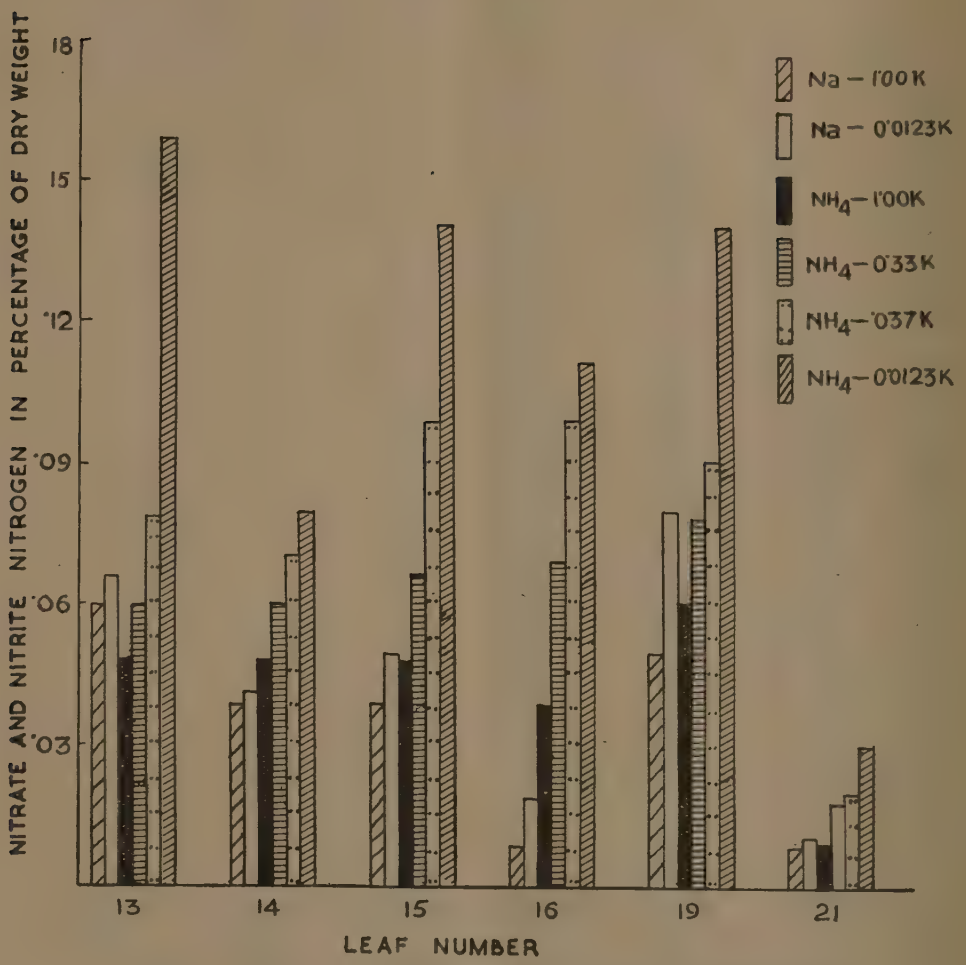

FIG. 12. Relation between nitrate and nitrate nitrogen and potassium supply

TABLE V

Analysis of ears

(*Nitrogen fractions expressed in percentage of dry weight*)

Treatment	Total	Non-Protein	Protein	Total Amino	Amide	Amino acid	Nitrite and nitrate	Ammonia
	N	N	N	N	N	N	N	N
NO_3 with 1K	1·2493	0·1457	1·1036	0·0143	0·0003	0·0140	0·0112	0·0068
NO_3 with 1/81K	1·0087	0·1268	0·8819	0·0301	0·0081	0·0220	0·0293	0·0087
NH_4 with 1 K	1·9274	0·3095	1·6179	0·0067	0·0001	0·0066	0·0097	0·0071
NH_4 with 1/3 K	0·9893	0·2364	0·7529	0·0084	0·0004	0·0078	0·0123	0·0079
NH_4 with 1/27 K	0·8765	0·1673	0·7092	0·0095	0·0016	0·0079	0·0236	0·0088
NH_4 with 1/81 K	0·7214	0·1819	0·5395	0·0154	0·0069	0·0095	0·0392	0·0103

DISCUSSION

The variations in the supply of potassium produced a series of changes in the rice plant. More important effects of the deficiency have been reported briefly [Sircar and Datta, 1954]. These include dull green to yellowish green colour of foliage, rapid die-back of leaves, death of tillers, reduction of stem elongation and increased succulence of the plant. These external symptoms corresponded essentially to those described in literature [Richards and Templeman, 1936 ; Ecstein *et al.*, 1937 ; and Hambidge, 1941]. But the green colour of the rice leaves was noticed before chlorosis had ensued—a finding confirmed by Eaton [1952] in the potassium deficient leaves of sunflower plant. The bluish tinge observed by McMurtrey [1938] in the potassium-starved tobacco leaves was not noticed in the present investigation. A striking feature was the development of red colour in some deficient leaves and this might be due to the presence of phloroglucinol as mentioned by Hartt [1934] in potassium-starved sugarcane.

The water content increased progressively with decreasing levels of potassium. The same relationship was noted by Richards and Shih [1940]. From Fig. 2 it seems that the higher water content in the potassium deficient sodium nitrate set was presumably due largely to the osmotic effect of the sodium taken up. When potassium was low, barley took up a large amount of sodium (if available) and the water content increased. Most plants, on the contrary, absorbed very little sodium even when potassium was deficient and their water contents did not show a rise. Hence the fact that water content in rice under K-deficiency increased markedly in the presence of sodium, but only slightly if at all when it was absent, was a

strong evidence to show that, like barley, rice was capable of absorbing sodium in quantity. The high potassium plants of the ammonium nitrate series tended to have higher water contents than those of the sodium nitrate one. It could not be due to osmotic effects of salts but might depend on the condition that the ammonium plants generally had greater nitrogen contents and obviously a greater amount of protoplasm relative to carbohydrate in accordance with their greater nitrogen supply. The only exception occurred in the data for the 13th leaf, where the sodium nitrate series had the higher content of nitrogen ; but here again the water content data were also reversed, the sodium leaf being more succulent. Therefore, the correlation was preserved and the results of higher water contents in high potassium plants of ammonium nitrate series were presumably largely explained by the different carbohydrate-nitrogen balance in the two series.

At the highest level of potassium, vigorous vegetative growth occurred, and as the potassium supply was diminished a progressive reduction in height, tiller number and grain yield resulted. These effects were found to be statistically significant.

This work clearly showed that the absorption of nitrogen was dependent on potassium supply. As the external concentration of potassium was varied over a wide range, the uptake of nitrogen varied in the same way (Fig. 5). The results did not seem to agree with those obtained by Gildehaus [1931] for apples, Janssen and Bartholomew [1932] for cowpeas and sugar beets, Colby [1933] for French prune trees, Rippel, Behr and Meyer [1933] for potatoes, Hartt [1934] for sugarcane, Sideris and Young [1946] for *Ananas comosus* and Cooil and Slattery [1948] for guayule, but were in accord with those of Gregory and his school for barley and of Eaton [1952] for sunflower.

When the leaves from the plants of the present investigation were compared with those from the rice in soil culture by Sen [1946], it was found that the nitrogen content in sand culture was relatively higher than that from the soil plants. This showed that there was no shortage of nitrogen supply to these experimental plants and that rice plants could grow normally at lower nitrogen levels than that of this work. When potassium was plentiful, the ammonium plants benefited from the extra nitrogen. But at the lowest potassium level both height and tiller number were considerably greater in the sodium nitrate series than in the ammonium nitrate series. Hence, the same degree of potassium deficiency was much more detrimental to vegetative growth in the NH_4NO_3 series than the $Na\ NO_3$ one.

With barley, the specially adverse effect of potassium deficiency lasted for several weeks Richards [1947]. Eventually the plants grew much better and often displayed a remarkable improvement late in their life history. The initial adverse influence might be due to the rapid absorption of too much nitrogen (especially ammonium nitrogen) under potassium deficiency where the plants indicated breakdown of nitrogen metabolism. As a natural consequence, large accumulation of amino acid, amide and other nitrogen fractions took place. Gradually the situation got cleared up as the amount of nitrogen entering the plant diminished with time and

the soluble nitrogenous substances decreased very much in amount and the growth improved. It is to be decided whether rice exhibits similar phenomena. The data on nitrogen fractions, however, seemed to bear them out quite well. Protein was lower in the 0·01 K set than that in the corresponding deficient sodium set, and all the estimated soluble fractions (ammonia, amino, amide and nitrate) were generally considerably higher. Amino and amide nitrogen fell sharply in the late leaves to levels no higher than in the sodium plants, so that the conditions might well have been on the mend at that time. This conclusion was perhaps confirmed by the grain data, for the 0·01 K ammonium plants apparently gave a larger yield than 0·01 K sodium plants.

It is generally believed that nitrate is converted to ammonia before it can enter into the metabolic machine. The prime effect on potassium deficiency of nitrogen metabolism was largely the accumulation of ammonium nitrogen. When amino acids and amide piled up owing to protein hydrolysis or failure of protein synthesis or both accompanied by continued absorption of nitrogen, there existed a high concentration of the end products of the reaction between ammonia and organic acids. By the law of mass action one would expect ammonia to accumulate and a similar law might be expected to hold good for the enzyme systems as Wadleigh [1949] claimed the possible role of potassium to be conditioning the action of specific enzymes. Since it accumulated by the law of mass action or its equivalent, we might expect its precursor to accumulate in its turn. Thus we found that nitrate and possibly nitrite accumulated in the rice plant.

Protein nitrogen was also affected by potassium supply in the same way as total nitrogen. As the concentration of potassium was lowered, there was a decrease in the quantity of protein nitrogen. This decrease in protein nitrogen was associated with the accumulation of amino acid and amide. This indicated that potassium was directly responsible for the synthesis of proteins.

According to Nightingale *et al.*, [1928], potassium is necessary for protein synthesis. But Gregory [1937] and Richards [1947] have stated that potassium is involved in protein synthesis and is indispensible to the maintenance of protoplasmic organisation. In its deficiency, protoplasm disintegrates with resultant hydrolysis of proteins and large accumulation of amino acids [Richard and Berner, 1954]. This often leads to early death of the leaves and tillers (a fact also corroborated by the present investigation). On the other hand, Wall [1940] has suggested that potassium may act as a catalyst in the condensation of amino acids to proteins. In tomato plants, he has found that in the early phase of potassium deficiency there is the accumulation of soluble nitrogen fractions and has admitted that proteolysis cannot account for their accumulation at this stage. He has observed that proteolysis takes place in the later phase of potassium deficiency when amino acids and amide increase. In the rice plant, proteolysis might account for the increase of amide and total amino nitrogen in the early phase of potassium deficiency. The necessity of potassium in protein synthesis was also evident in the fruiting stage.

SUMMARY

A sand culture experiment was carried out in which rice plant, var. *Bhasamanik*, was grown at different levels of potassium using sodium nitrate and ammonium nitrate as sources of nitrogen. The external symptoms of potassium deficiency were noted in the leaf, stem and ear.

With an ample supply of potassium ammonium plants benefitted from the extra nitrogen, but at the lower level of potassium, growth was better in sodium nitrate series than in the ammonium nitrate plants, suggesting that the same degree of potassium deficiency was much more harmful to growth in the ammonium nitrate series than in the sodium nitrate one. Decreased supplies of potassium led to progressive reduction in height, tillering and yield.

The water content in the leaves increased progressively with decreasing levels of potassium supply in the sodium nitrate series but marked fluctuations occurred at different levels of ammonium nitrate plants.

The leaves of high potassium plants contained more total and protein nitrogen-amide, amino, nitrate and nitrite nitrogen were, however, greater in low potassium plants. The potassium deficient plants died prematurely on account of ammonium poisoning. The role of potassium on growth and nitrogen metabolism of the plant was discussed.

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STUDIES ON CERTAIN ASPECTS OF GERMINATION OF SEEDS IN CASHEW (*ANACARDIUM OCCIDENTALE* LINN)

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THE cashew (*Anacardium occidentale* L.) is now one of the major dollar earning crops of India and large scale extension of area under it is under way. Cashew has been a neglected crop so far, being grown on wastelands and in soils considered generally unfit for other crops. Moreover, there has been no serious attempt to determine the optimum conditions for its satisfactory growth and performance. Its hardy nature and characteristic adaptability to diverse soil and climatic conditions, have been to an extent responsible for a misconception, that the crop does not need much attention. Several views are, however, prevalent among the growers on the different aspects of the cultivation which being as divergent as they are vague have little practical utility. In the absence of concrete evidence in support of the several traditional belief in the different tracts, cultivators have necessarily had to adopt indifferent methods of culture resulting in uneconomic returns and unstandardized produce. One of such major aspects concerns the kind of seed and the method of sowing which are as important in cashew cultivation as in any other crop, not only for satisfactory crop performance but for avoiding wastage of labour and money. Observational studies on certain aspects of the germination of seeds in cashew were initiated at the Central Cashewnut Research Station, Mangalore, in 1954.

Review of Literature

At the Agricultural Research Station, Taliparamba, trials in 1944-45 showed that sowing the seed with the suture slanting and at two inches below the surface of the soil was the best in respect of germination and freedom from pests. Only two treatments, viz. one inch and two inches, however, were considered. At the Agricultural Research Station, Nileshwar in 1937-38, it was found that (i) for seed purposes, large sized and heavy nuts were to be preferred and (ii) there was no difference in germination, (*a*) between partially mature and fully mature seeds, (*b*) between nuts sown with and without apple, and (*c*) between three lots of seeds, collected from different localities representing different soil conditions. Patel [1932] recommends plump, well shaped and medium sized heavy nuts obtained from fully ripe fruits as best for seed purposes. A note on cashewnuts [1946] mentions that unless the nuts are planted deep (at least 5 in.) they are frequently destroyed by vermin insects, etc. Morada [1941] and Paul [1933] though dealing in detail with cashew culture, do not make any mention of the type of seed to be selected, nor of the method of sowing.

Material and Methods

The seeds were collected from selected trees at the Agricultural Research Station, Taliparamba (Malabar) and from plantations in the neighbourhood of the Central Cashewnut Research Station, Mangalore during 1954 and 1955 seasons.

Influence of period of collection of seeds on germination

Mature seeds, harvested from a selected tree in fortnightly batches from February to April 1955 (five batches) were sown on the 20th June 1955, at the rate of 40 seeds per treatment, in a randomised trial replicated four times. The seeds were sown one foot apart either way, with the stalk end up and the suture in a slanting position at 45° to the surface of the soil. The percentage of germination of the seeds in each batch was recorded.

Influence of stage of maturity of seeds on germination

Seeds were collected from a single tree of a type bearing red apples to represent six stages of maturity as detailed below :

1. Fully mature seed, pericarp hard, oily, grey ; kernel sweet, oily, testa thin ; apple deep red, larger than the nut ; seeds collected after dropping from tree.

2. Fully mature seed, pericarp hard, oily, grey ; kernel sweet, oily, testa thin ; apple red, larger than the nut ; seeds harvested from the tree.

3. Fully mature seed, pericarp hard, oily, light grey ; kernel sweet, oily, testa thin ; apple green, larger than the nut ; seeds harvested from the tree.

4. Seed not fully mature, pericarp slightly yielding to prick, less oily, light grey ; kernel sweet, less oily, testa thin ; apple green, larger than the nut ; seeds harvested from the tree.

5. Seed not fully mature, pericarp soft, yielding to prick, without oil, greenish grey ; kernel slightly sweet, without oil, testa thick ; apple green, smaller than the nut ; seeds harvested from the tree.

6. Seed not fully mature, pericarp soft, easily yielding to prick, without oil, green and glossy ; kernel insipid, without oil, testa very thick ; apple green, smaller than the nut ; seeds harvested from the tree.

The first series at the rate of 20 seeds per treatment was dried in the sun for two days, immediately after collection and sown on the 27th March 1955 in flat nursery beds one foot apart at a depth of two inches and with the stalk end facing upwards and the suture in a slanting position at 45° to the surface of the soil. The beds were watered daily till the break of the monsoon in June, 1955.

A second series of seeds after sun-drying for two days was stored in stoppered glass bottles for 77 days and sown on the 14th June, 1955 in flat nursery beds as in the previous batch. No watering was done at any stage as the seeds had the benefit of rains. The percentage of germination of the seeds under each treatment in the two series was recorded.

Influence of size, weight and density of seed on germination

The following categories of seeds collected from a single tree were sown on the 15th June, 1955, at a depth of two inches with the stalk end facing upwards and the suture in a slanting position at 45° to the surface of the soil. Forty seeds were sown per treatment in a randomised layout replicated four times.

Size

(*i*) Big (3·2×2·8 sq. cm. to 3·5×3·0 sq. cm.)
(*ii*) Medium (2·8×2·5 sq. cm. to 3·1×2·7 sq. cm.)
(*iii*) Small (2·5×2·0 sq. cm. to 2·7×2·4 sq. cm.)

Weight

(*i*) Heavy (50 seeds per pound)
(*ii*) Medium (60 seeds per pound)
(*iii*) Light (80 seeds per pound)

Density

(*i*) Seeds sinking in water
(*ii*) Seeds floating in water

A separate group constituting malformed seeds was also sown along with the others.

Influence of the planting position of seeds on germination

Seeds of uniform size, from a selected tree at the Agricultural Research Station, Taliparamba, were sown on the 4th December 1954 at a depth of two inches in the following six positions at the rate of 50 per treatment in raised beds the soil being made up of red laterite earth.

1. Stalk end facing upwards
2. Stalk end downwards
3. Suture (sinus) upwards
4. Suture (sinus) downwards
5. Flat on the sides
6. Stalk end up and inclined at 45° to the base (slanting position)

The beds were watered at intervals of three days throughout the period. Data on the percentage of germination, rate and time of appearance and shrivelling of the cotyledons above the surface under each treatment were collected.

Influence of depth of sowing of seeds on germination

Seeds of uniform size from a selected tree in a neighbouring plantation were sown in beds at six different depths ranging from one to six inches, with the stalk end facing upwards and the suture in a slanting position at 45° to the surface of the soil at a spacing of six inches, in rows two feet apart. The treatments comprising 60 seeds each were randomised and replicated six times. No watering was done at any stage as the seeds had the benefit of the monsoon rains.

Results

The percentage of germination recorded in five batches of seeds collected at fortnightly intervals from February to April, 1955, and sown on the 20th June, 1955 are given in Table I.

Table I

Influence of period of seed collection on germination

Serial No.	Seed collection	Percentage of germination
1	Seeds collected during the first fortnight of February 1955	98
2	Seeds collected during the second fortnight of February 1955	90
3	Seeds collected during the first fortnight of March 1955	100
4	Seeds collected during the second fortnight of March 1955	98
5	Seeds collected during first fortnight of April 1955	95

The differences between the treatments were not statistically significant at 5 per cent level. The study, therefore, does not bear out the general belief that the seeds collected from the early harvests of the season are superior in germination to those collected later.

The results of the study of influence of maturity of seeds on germination are given in Table II.

Table II

Influence of maturity of seeds on germination

Stage of maturity	Percentage of germination when sown in	
	March '55	June '55 after storage
Seed fully mature, collected after dropping from the tree	95	100
Seed fully mature, pericarp grey, apple red, seeds harvested from the tree	95	100
Seed fully mature, pericarp light grey, apple green, seed harvested from the tree	90	100
Seed not fully mature, pericarp, slightly yielding to prick, testa thin, apple larger than the nut	100	100
Seed not fully mature, pericarp, soft, testa thick, apple smaller than the nut	100	100
Seed not fully mature, pericarp soft, testa very thick, apple smaller than the nut	100	95

The data in Table I show that no particular advantage is to be gained, as popularly claimed, in selecting nuts that are not fully mature for sowing purposes. The storage of seeds for about two and a half months had also not appreciably influenced the germination of the seeds.

Growth measurements of the first batch of seedlings at the end of four months and of the second batch at the end of two months also failed to reveal any perceptible differences between the several treatments.

The data on influence of size and weight of seeds on germination recorded at the end of 40 days after sowing are given in Table III.

TABLE III

Influence of size and weight of seeds on germination

Serial No.	Treatment	Percentage of germination
1	Heavy (50 seeds per lb.)	37·5
2	Medium (60 seeds per lb.)	70·0
3	Light (80 seeds per lb.)	52·5
4	Big (3·2×2·8 to 3·5×3·0 sq. cm.)	55·0
5	Medium (2·8×2·5 to 3·1×2·7 sq. cm.)	70·0
6	Small (2·5×2·0 to 2·7×2·4 sq. cm.)	50·0
7	Seeds sinking in water	47·5
8	Seeds floating in water	35·0
9	Malformed	7·5

Although the above data indicate that the medium seeds both by weight and size are preferable to the other classes of seeds, in respect of germination, it seems, however, necessary to consider a larger population to arrive at conclusive results.

Germination counts were recorded at the end of 40 days after sowing under six treatments. The data on the time of appearance of cotyledons above the soil surface under each treatment and the time taken for the cotyledons to shrivel up

completely after germination were also recorded. The results are summarized in Table IV.

TABLE IV

Influence of the planting position of seeds on germination

Treatment	Percentage of germination	Percentage of seedlings in which cotyledons appeared above the surface	No. of days for the appearance of cotyledons	No. of days for the cotyledons to shrivel up	Nature of plumule growth
Stalk end upwards	52	20	26	40	Straight
Stalk end downwards	12	..	..	..	Bent and curved
Suture upwards	28	4	26	38	Straight
Suture downwards	28	20	27	40	do.
Flat	40	32	26	38	do.
Stalk end upwards in a slanting position.	44	2	26	38	do.

It is seen that (*i*) the maximum germination is obtained when the seeds are sown with the stalk end facing upwards but a larger number of cotyledons appear above the surface as compared to the treatment in which the seeds were sown in a slanting position under which a germination of 44 per cent has been recorded with only two per cent of the seedlings having the cotyledons exposed.

(*ii*) Sowing seeds with the stalk end downwards is definitely detrimental to proper germination. The growing stem is also crooked.

(*iii*) Sowing with the suture upwards or downwards is not conducive to good germination while seeds sown flat on the sides, though registering a satisfactory germination, suffer from the disadvantage of having nearly a third of the seedlings with exposed cotyledons.

(*iv*) The slanting position of the seed with the stalk end upwards, which possesses both the favourable features viz. satisfactory germination and a relatively low percentage of cotyledon emergence, therefore, is to be deemed as the best method of sowing.

Observations on influence of depth of sowing of seed on the germination of seeds and the emergence of cotyledons were recorded in respect of the seeds sown at six different depths (Table V).

TABLE V

Influence of depth of sowing of seed on germination

Depth of sowing (inches)	Mean No. of days for the first signs of germination from the date of sowing	Mean No. of days for complete germination	Percentage of germination	Percentage of seedlings in which cotyledons appeared above the surface
1	16	17	51·7	51·7
2	18	20	60·0	5·0
3	21	28	48·3	*Nil.*
4	26	27	23·3	*Nil.*
5	26	28	11·7	*Nil.*
6	43	44	1·7	*Nil.*

The following is a summary of the statistical analysis of the data on the percentage of germination :—

Particulars	Treatment (depth of sowing in inches)					
	One	Two	Three	Four	Five	Six
	(1)	(2)	(3)	(4)	(5)	(6)
Mean percentage of germination	51·7	60·0	48·3	23·3	11·7	1·7
Significance by 'F' test (p= 0·05)			Significant			
Critical difference	16·3	..	..	..	..	..
Standard error	5·59	..	..	..	..	..
Conclusion	(2)	(1)	(3)	(4)	(5)	(6)

The above data show that sowing seeds 1-3 in. deep is significantly superior to the other treatments in regard to the percentage of germination. Between the first three treatments, however, although no significant difference is seen, it is preferable to sow the seeds from 2-3 in. deep to avoid emergence of the cotyledons above the ground. In 50 per cent of the seeds sown at 6 in. depth, germination had been initiated but due possibly to the depth, the delicate plumule could not thrust itself up resulting in the death of the seedlings. Similar observations were recorded in the seeds sown 4-5 in. deep, of which 30 and 33 per cent respectively failed.

Discussion

The results of the trials under report emphasize in general the need for care and attention in the selection of proper seed material and the method of sowing. They also serve to demonstrate the extent to which loss could occur through indifferent methods of seed selection and sowing but how easily such mistakes could be avoided.

The trials have shown no appreciable difference in the germination of seeds collected during the different periods of the cropping season. At any rate, seeds collected at the end of the season have proved to be quite as good as those collected earlier.

The studies on the influence of the relative maturity of the seeds on germination fail to lend support to a belief that seeds not fully mature are superior to those fully mature. These results agree with the findings at Nileshwar [1954]. Further trials under field conditions seem warranted to verify the supposed difference in vigour, yield, etc. of trees raised from these two categories of seeds. Until definite information on this aspect is available there seems to be no valid justification for any departure from the normal procedure of selecting nuts fully mature, that have either dropped or have been harvested from the tree.

Medium seeds, both by weight and size have indicated superiority over heavy and large or light and small seeds. Further studies appear necessary to confirm the results.

Sowing seeds in certain definite positions, as with the stalk end facing upwards and in a slanting position has been proved to be preferable to any other method, in view of its double advantage of recording a satisfactory germination and having the minimum number of seedlings with fleshy cotyledons exposed. This is a point of great significance in forest areas where the damage to the seedlings by pests like birds, monkeys, jackals and rodents assumes alarming proportions. Another noteworthy result is the large difference of 40 per cent in the rate of germination of seeds sown with the stalk end upwards and in the reverse direction, which indicates the scope for saving money and labour on seeds and sowing, particularly in large scale extension work. Sowing seeds with the suture upwards or downwards is not

conductive to satisfactory germination while the placement of the seed flat on its side is again undesirable in view of a large proportion of the cotyledons emerging above the ground surface. The cotyledons emerge out of the seedlings only from the 25th day after sowing and shrivel up at the end of 40 days, indicating thereby that whatever steps that are taken to protect the seedlings from pests may be restricted to this period after which due to the absence of the edible cotyledons, the seedlings cease to attract the pests. From the point of view of nursery requirements, especially for budding and grafting operations, sowing seeds with the stalk end downwards seems to be undesirable as in that position of the seed, the growing stem tends to be crooked. In all other positions, the stem is straight and, therefore, easy to work.

The results under the trials on depth of sowing, agree with those conducted at the Agricultural Researcl. Station, Taliparamba [1946], although in the latter only two treatments were employed, viz. one inch and two inches, as against six in the present investigations. Sowing seeds two inches deep has been found to be best. Seeds sown deeper than three inches have recorded very poor germination. Examination of these seeds showed that although germination has been initiated, the delicate plumule has not been able to thrust itself above the soil surface. In forest areas subject to depredations by pests, it seems, therefore, safe to sow seeds 3 in. deep for satisfactory germination and to ensure at the same time that the fleshy cotyledons do not emerge out. It is also clear that sowing too deep, in an anxiety to aim at a thorough freedom from pests is also not a wise step in view of the low germination.

On the basis of the results of the investigations outlined above, it seems possible to infer that medium sized seeds, harvested from the tree when fully mature, during any part of the cropping season and sown 2-3 in. deep with the stalk end facing upwards and in a slanting position may be considered as the optimum.

Summary

Trials were conducted at the Central Cashewnut Research Station, Mangalore, during 1954-55 to determine the optimum kind of seed material, time of collection of seeds from the parent tree and the method of sowing, for securing satisfactory germination. The following are the main results.

1. There is no appreciable difference in germination of seeds collected during five different periods in the cropping season and sown simultaneously.

2. Seeds not fully mature have no particular advantage over those fully mature as generally believed in same tracts.

3. Sowing the seeds with the stalk end facing upwards and in a slanting position offers the best means of securing a high percentage of germination and freedom from pests that attack the fleshy edible cotyledons.

4. Seeds sown two or three inches deep have given the best results; sowing deeper than three inches has been deterimental to proper germination.

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SEED-TREATMENT FOR BREAKING DORMANCY OF POTATOES

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POTATO tubers remain dormant or pass through a rest period for two months or more after they are harvested and there is usually a varietal difference in this period. The tubers, during this period, fail to sprout even if optimum conditions for sprouting are provided. This is an advantage as freshly harvested potatoes can be stored and used for consumption over a peroid of time without much loss in quantity or quality, which the sprouting will otherwise cause. From the seed point of view, however, dormancy presents a serious problem as the tubers from a freshly harvested crop cannot be used as seed immediately or within the dormancy period; if the dormant tubers are planted, there is poor and delayed germination resulting in gappy stand and reduced yield of the crop.

In northern India, the potato crop in the higher hills is generally harvested in September and October and in the plains the main planting is done in October-November. The seed harvested in the hills, although of a better quality because of its relative freedom from diseases, cannot be planted immediately in the plains during the main crop season, because of its dormancy. It has, therefore, to be planted as a second crop, late in the season in December-January or the seeds from lower elevations where the crop is harvested earlier has to be used in the plains. In both these cases, there are certain disadvantages. Late planting can be done only in certain areas where the season is longer as in the western U. P. and Punjab. Moreover, late planting in the plains invariably yields poorly and is exposed to the spread of virus diseases through the agency of aphids which appear in large numbers late in the season. The seed from lower elevations is not generally healthy as, in addition to viruses, it is likely to be infected with bacterial wilt and brown rot and nematodes. Again, in certain areas as in the Nilgiris, where successive potato crops are taken in a year, it becomes necessary to use the seed of the previous crop for planting the immediately succeeding crop. In this case also, the tubers, because of their dormancy are not suitable for immediate planting. In such cases it will be an advantage if some simple treatment can be given to the seed to break its dormancy. With a view to devising a simple method of seed-treatment, which would reduce the period of dormancy in potatoes so that they could be used as seed soon after harvest, experiments were undertaken at the Central Potato Research Institute, Patna.

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Review of Literature

Work on the inducement of sprouting in dormant seed potatoes has received considerable attention in the U.S.A. since the early part of the present century. McCallum [1909] discovered that exposure of tubers to vapours of ethyl bromide, carbon tetrachloride, or ethylene dichloride among others, was effective in breaking dormancy. Appleman [1916] stimulated sprouting by peeling dormant tubers or by wrapping them in cotton wool soaked in hydrogen peroxide. Rosa [1923] obtained successful results with soaking cut pieces in oxidizing agents such as sodium nitrate and potassium permanganate. Extensive studies with 224 chemicals carried out by Denny [1926, a] led to the discovery of ethylene chlorohydrin and thiocyanate of sodium or potassium as the most effective dormancy-breaking chemicals. Thiourea not only induced prompt sprouting but also counteracted apical dominance. Ammonium thiocyanate also gave satisfactory results [Denny, 1926, b]. Temperature played an important role on the effect of chemical treatment [Denny, 1928].

A few investigations on shortening dormancy have also been carried out in India. Pal and Pushkarnath [1938] reported that peeling of tubers overcame dormancy. They also found that ethylene chlorohydrin, though generally effective gave varying results. They, however, concluded that such methods of breaking the rest period of potato tubers were unlikely to be adopted by commercial growers but they were of value to potato breeders and geneticists. Dutta and Thakurta [1948] observed that 1 per cent ethylene chlorohydrin or 2 per cent thiocyanates of sodium, potassium or ammonium was more effective than lower doses, and cut or immature tubers showed better response to treatment than whole or mature ones. Sen Gupta and Chattopadhya [1954] recorded improvement in germination by thiocyanates of ammonium and potassium. The treatment with these chemicals, however, increased the rottage of tubers. In these experiments, whole tubers responded better than cut tubers, contrary to the findings of other workers. Ranjan and Kaur [1954] found that N ethylene chlorohydrin and 1 per cent ammonium thiocyanate influenced growth as well as respiratory activity favourably but lower doses (0·1 N ethylene chlorohydrin or 0·5 per cent ammonium thiocyanate) were without effect. Two per cent thiocyanate depressed germination as well as the respiration rate. Experiments carried out at Nanjanad (Nilgiris) by Saptharishi and Azariah [1953] have shown that exposure of dormant tubers to carbon-bi-sulphide at 1 oz. per 32 cu. ft. (800 lb. capacity) in air-tight containers for 10 days, followed by 7 days' storage under straw, induced early sprouting. The treatment is also reported to be practised by growers in the Nilgiris. However, this method is time-consuming and not safe in the hands of the ordinary growers, as carbon-bi-sulphide is inflammable.

It is evident from the foregoing review that further work was necessary to evolve a safe and simple method for breaking dormancy of potato tubers in order that it may be adopted by growers.

MATERIAL AND METHODS

Three series of experiments were conducted during the seasons commencing from 1952-53. In the first two series, seed potatoes of the varieties, D. R. R. (Darjeeling Red Round) and Up-to-date obtained from the harvest in the hills were used while for the third series, the seed of a hybrid, O. N. 249, harvested at Patna, was used. In all the cases, the tubers were cut into pieces before treatment with the chemicals.

Series I

Ethylene chlorohydrin, thiourea and ammonium thiocyanate were chosen for the study. A 'dip' treatment method was adopted with ethylene chlorohydrin and a 'soak' treatment with thiourea and ammonium thiocyanate. The dip treatment consisted of dipping the seed material momentarily in an aqueous solution of the chemical followed by storage of the treated material in an air-tight container for 24 hours. The soak treatment consisted in soaking the seed material for one hour in an aqueous solution of the chemical, prior to planting. The following treatment were tried singly as well as in combination :

(*a*) Dip treatment in 0, 1·5, 3·0 and 4·5 per cent concentrations of ethylene chlorohydrin ;

(*b*) Soak treatment in 0, 1 and 2 per cent concentrations of each of thiourea and ammonium thiocyanate.

The total number of treatment combinations included in the experiment was 24, of which four were dummy treatments (0 concentration of thiourea being identical with 0 level of thiocyanate for each concentration of ethylene chlorohydrin).

During 1952-53 season, seed potatoes of D. R. R. harvested at the Potato Multiplication Substation, Bhowali (5,000 ft. elevation) in the middle of September 1952, were obtained and tubers of uniform size were selected and cut into two halves. The cut pieces were divided into 24 lots of 320 pieces each, and to each lot, one of the above 24 treatments was given. After treatment, the material was planted in 4 randomised blocks on 16-12-1952. The individual plots measuring 6 ft. × 6 ft., consisted of 4 rows planted with 20 seed pieces in each row. Germination counts were taken during January 5 to January 27, 1953 on alternate days. The yield of each experimental plot (3 ft. × 6 ft.) after rejecting border rows, was recorded on March 3, 1953.

During 1953-54, the above experiment was repeated with seed of D. R. R. and Up-to-date obtained from Bhowali and Kufri but due to a heavy infestation of the crop with cutworms (*Agrotis ypsilon*) and accidental flooding of a part of the area, the experiment was discontinued.

The experiment was repeated in 1954-55 with seed of D.R.R. and Up-to-date harvested at the Potato Certification Substation, Kufri, (elevation 9,000 ft.) in the middle of October and middle of September respectively. After the same chemical treatments as in 1952-53, four replicates with D. R. R. and two replicates with Up-to-date were planted in the field on the 23rd and 25th November 1954 respectively. Each plot measuring 6 ft. × 6 ft. consisted of four rows of 10 pieces each. Germination counts were recorded at 2 to 3 days intervals during the period December 13, to January 12, 1955. The yield of potatoes from each experimental plot (3 ft. × 6 ft.) was recorded on March 19, 1955 for D. R. R. and on March 7 for Up-to-date.

Series II

The chemicals used in this series of experiments conducted during 1954-55, were the same as in the previous series, but the concentrations were slightly modified to find the optimum concentration. Again the seed potatoes of the two varieties, D. R. R. and Up-to-date, obtained from the hills were used in the experiments.

The treatments, numbering 20, consisted of all combinations of—

(*a*) Dip treatment in 0, 0·5, 1·0 and 1·5 per cent concentrations of ethylene chlorohydrin ;

(*b*) Soak treatment in 0, 1 and 2 per cent concentrations of thiourea and 0·5 and 1·0 per cent concentrations of ammonium thiocyanate.

After appropriate chemical treatments, the seed pieces were planted in the field with 6 replications under each variety on November 19, 1954. Each plot consisted of 6 rows and measured 9 ft. × 6 ft. Fifteen and 10 seed pieces of D. R. R. and Up-to-date respectively were planted in a row. Germination counts were recorded during 8th December to 24th December, 1954 on alternate days. Yield data were recorded for each experimental plot (6 ft. × 6 ft.) on March 17, 1955 for D.R.R. and on March 6, 1955 for Up-to-date.

Series III

Under this series, only one experiment was conducted during 1953-54 with seed of O. N. 249 harvested at the C.P.R.I., Patna, on December, 1953. One hundred and twenty five tubers of uniform size were selected and divided into 5 lots of 25 tubers each for the following treatments :

(1) O : Tubers cut into quarters but not treated

(2) E : Tubers cut into quarters, dipped in 3 per cent aqueous solution of ethylene chlorohydrin and stored for 24 hours in a closed container

(3) T : Tubers cut into quarters and soaked in 1 per cent aqueous solution of thiourea for one hour

(4) TE : Tubers cut into quarters and soaked in 1 per cent aqueous solution of thiourea for one hour and dipped in ethylene chlorohydrin (3 per cent) and stored for 24 hours in closed containers.

(5) ET : Tubers cut into halves, treated with ethylene chlorohydrin (3 per cent) stored for 24 hours in closed container and split into two equal lots for : (i) treatment with thiourea (1 per cent) followed by cutting into quarters and (ii) cutting first into quarters, followed by treatment with thiourea (1 per cent).

The treatments were given on the 7th and 8th December 1953 and seed pieces were planted in the field on December 8th, 1953. One hundred seed pieces (quarters) of each treatment were planted in each plot without replication. Counts of emergence were recorded from 26th December to 21st January 1953 at 4-day intervals. Yield data were not recorded.

In the experiments under series I and II, the treatment effects were studied on (1) the plant number recorded on the last date of observation expressed as a percentage of the seed pieces planted, (2) the 'rate index' which is a measure of the speed of emergence, calculated according to the method described by Bartlett [1937] and (3) the yield of potatoes. Statistical analyses and tests of significance were performed wherever necessary.

EXPERIMENTAL RESULTS

It may be noted that the seed material used in the experiments was at different stages of dormancy, depending on variety and the interval between the harvest in the hills and planting in the plains. This should be borne in mind in the interpretation of treatment effects in breaking dormancy.

Series I

The effects of the various seed treatments on sprout emergence, rate index and yield of potatoes are given in Table I.

It will be seen from the results that :

(i) dip treatment with 1·5—4·5 per cent ethylene chlorohydrin increased the stand, the rate index and the yield in both the varieties although the magnitude of effects and the optimum concentration depended on the stage of dormancy of the seed tubers. In the case of the variety D.R.R., which is known to have a longer period of dormancy, treatment with ethylene chlorohydrin at 1·5—4·5 per cent gave progressive increases in germination, rate index and yield, although the rate of improvement decreased with increase in concentration. In 1954-55 when the D.R.R. seed was treated about a month after harvest and was fully dormant, the chemical was more effective than in 1952-53 when the seed was treated three months after harvest and was only partially dormant. In the case of the variety Up-to-date, which has a short dormancy of about two months duration, the magnitude of the effects of the treatment were not marked as the seed at the time of treatment had already passed through a considerable portion of its rest period,

(ii) with both the varieties, soak treatment with 1 per cent thiourea was nearly as effective as dip treatment with 1·5 per cent ethylene chlorohydrin in regard to the percentage stand, the speed of emergence and the yield, but 2 per cent thiourea was very much less effective than 1 per cent thiourea or 1·5 per cent ethylene chlorohydrin, particularly in respect of yield of potatoes.

TABLE I

The effect of seed treatment with chemicals on sprout emergence, rate index and the yield of potatoes (Series I)

Variety Source of seed Date of harvest Date of planting after treatment Year	D. R. R. Bhowali Middle of September, 1952 16-12-1952 1952-53				
Treatments	0	Thiourea 1 per cent	2 per cent	Ammonium thiocyanate 1 per cent	Ammonium thiocyanate 2 per cent
	Percentage emergence 42 days after planting				
Ethylene chlorohydrin—					
0	42·5	71·5	72·5	63·7	51·9
1·5 per cent	66·1	79·7	87·7	79·4	57·5
3·0 per cent	72·4	80·6	92·5	74·7	56·9
4·5 per cent	75·9	75·6	87·2	83·1	53·4
	Rate Index (Bartlett)				
Ethylene chlorohydrin—					
0	0·455	0·590	0·550	0·554	0·399
1·5 per cent	0·605	0·708	0·667	0·752	0·517
3·0 per cent	0·612	0·710	0·679	0·667	0·573
4·5 per cent	0·623	0·692	0·688	0·764	0·639
S. E.	±0·017	±0·024	±0·024	±0·024	±0·024
C. D.	(*a*)0·049	(*b*)0·060	(*c*)0·069		
	Yield in maunds per acre				
Ethylene chlorohydrin—					
0	35·1	59·5	49·1	59·5	39·7
1·5 per cent	75·5	84·6	72·9	87·8	42·9
3·0 per cent	83·0	104·9	84·9	77·9	56·4
4·5 per cent	88·3	95·5	97·9	86·2	47·7
S. E.	± 6·8	±9·6	±9·6	±9·6	±0·6
C. D.	(*a*)19·1	(*b*)23·4	(*c*)27·0		

C. D. (*a*)—Least significant difference to test the significance of comparisons between varying levels of ethylene chlorohydrin at 0 level of soak treatment;

C. D. (*b*)—Least significant difference to test the significant of comparisons between levels of thiourea or thiocyanate (excluding 0 level) alone or in combination with ethylene chlorohydrin;

C. D. (*c*)—Least significant difference to test the significance of comparisons between any level of ethylene chlorohydrin (0, 1·5, 3, 4·5 per cent) without soak treatment and a soak treatment alone or in combination with ethylene chlorohydrin (1·5, 3, 4·5).

TABLE I—contd.

The effect of seed treatment with chemicals on sprout emergence, rate index and the yield of potatoes (Series I)—contd.

Variety Source of seed Date of harvest Date of planting after treatment Year	D.R.R. Kufri Middle of October, 1954 23-11-1954 1954-55				
Treatments		Thiourea		Ammonium Thiocyanate	
	0	1 per cent	2 per cent	1 per cent	2 per cent
	Percentage Emergence 41 days after planting				
Ethylene chlorohydrin—					
0	6·3	61·9	53·7	42·5	11·9
1·5 per cent	58·1	91·9	77·5	68·1	22·5
3·0 per cent	75·3	88·7	78·1	76·9	28·7
4·5 per cent	85·0	91·9	90·0	78·7	38·7
	Rate Index (Bartlett)				
Ethylene chlorohydrin—					
0	0·399	0·535	0·422	0·556	0·609
1·5 per cent	0·616	0·866	0·737	0·634	0·346
3·0 per cent	0·764	0·758	0·727	0·743	0·478
4·5 per cent	0·822	0·893	0·783	0·737	0·516
S. E.	±0·042		±0·060		
C. D.	(*a*)0·120	(*b*)0·147	(*c*)0·170		
	Yield in maunds per acre				
Ethylene chlorohydrin—					
0	57·4	143·7	94·4	97·2	76·4
1·5 per cent	154·0	181·9	143·3	160·3	62·7
3·0 per cent	154·0	128·5	141·5	159·9	100·30
4·5 per cent	188·0	164·5	190·4	177·4	112·0
S. E.	±13·1	±18·5			
C. D.	(*a*)37·0	(*b*)45·3	(*c*)52·3		

C. D. (*a*)—Least significant difference to test the significance of comparisons between varying levels of ethylene chlorohydrin at 0 level of soak treatment;

C. D. (*b*)—Least significant difference to test the significance of comparisons between levels of thiourea or thiocyanate (excluding 0 level) alone or in combination with ethylene chlorohydrin;

C. D. (*c*)—Least significant difference to test the significance of comparisons between any level of ethylene chlorohydrin (0, 1·5, 3, 4·5 per cent) without soak treatment and a soak treatment alone or in combination with ethylene chlorohydrin (1·5, 3, 4·5 per cent).

TABLE I—contd.

The effect of seed treatment with chemicals on sprout emergence, rate index and the yield of potatoes (Series I)—contd.

Variety Source of seed Date of harvest Date of planting after treatment. Year	Up-to-date Kufri Middle of September, 1954 25-11-1954 1954-55				
Treatments	0	Thiourea		Ammonium Thyiocyanate	
		1 per cent	2 per cent	1 per cent	2 per cent
	Percentage emergence 48 days after planting				
Ethylene chlorohydrin—					
0	75·0	91·3	90·0	88·8	58·8
1·5 per cent	83·0	97·5	88·8	82·5	67·5
3·0 per cent	81·5	88·8	95·0	81·3	87·5
4·5 per cent	81·8	87·5	97·5	78·8	70·0
	Rate Index (Bartlett)				
Ethylene chlorohydrin—					
0	0·641	0·796	0·515	0·649	0·408
1·5 per cent	0·789	0·817	0·746	0·718	0·626
3 0 per cent	0·755	0·770	0·719	0·695	0·719
4·5 per cent	0·731	0·794	0·693	0·689	0·711
S. E.	±0·022		±0·031		
C. D.	(*a*)0·065	(*b*)0·079	(*c*)0·091		
	Yield in maunds per acre				
Ethylene chlorohydrin—					
0	112	0·131	97	111	94
1·5 per cent	146	129	121	121	92
3·0 per cent	124	123	131	94	118
4·5 per cent	130	142	111	102	106
S. E.	±10·8	+15·4			
C. D.					

C. D. (*a*)—Least significant difference to test the significance of comparisons between varying levels of ethylene chlorohydrin at 0 level of soak treatment;

C. D. (*b*)—Least significant difference to test the significance of comparisons between levels of thiourea or thiocyanate (excluding 0 level) alone or in combination with ethylene chlorohydrin;

C. D. (*c*)—Least significant difference to test the significance of comparisons between any level of ethylene chlorohydrin (0, 1·5, 3, 4·5 per cent) without soak treatment and a soak treatment alone or in combination with ethylene chlorohydrin (1·5, 3, 4·5 per cent).

(iii) with partially dormant D.R.R. seed, 1 per cent ammonium thiocyanate had the same effect as 1 per cent thiourea in all respects but with fully dormant seed of D.R.R., the former was inferior to the latter in regard to stand and yield. With Up-to-date, 1 per cent thiocyanate improved the stand but this was not reflected in higher yield. Two per cent thiocyanate was much less effective than its lower concentration, with D.R.R. and was harmful with Up-to-date.

(iv) a combined treatment of 1 per cent thiourea and 1·5 per cent ethylene chlorohydrin was highly effective in the case of D.R.R. but the effect of the double treatment was not so marked on Up-to-date, as the seed had already lost much of its dormancy.

Series II

The results of the various seed treatments on sprout emergence, rate index, and the yield are given in Table II

TABLE II

The effect of seed treatment with chemicals on sprout emergence, rate index, and the yield of potatoes (Series II)

Variety Source of seed Date of harvest Date of planting after treatment Year	D. R. R. Kufri Middle October, 1954 November 11, 1954 1954-55				
Treatments		Thiourea		Ammonium Thiocyanate	
	1	1 per cent	2 per cent	0·5 per cent	1·0 per cent
	Percentage Emergence, 35 days after planting				
Ethylene chlorohydrin—					
0	12·4	68·9	63·0	57·6	47·0
0·5 per cent	29·1	83·7	50·9	73·6	59·1
1·0 per cent	41·4	82·0	56·4	79·8	67·4
1·5 per cent	37·0	86·1	73·0	68·0	78·0
	Rate Index (Bartlett)				
Ethylene chlorohydrin—					
0	0·349	0·428	0·403	0·413	0·295
0·5 per cent	0·436	0·622	0·500	0·558	0·409
1·0 per cent	0·447	0·682	0·419	0·665	0·441
1·5 per cent	0·427	0·696	0·537	0·624	0·446
S. E.	±0·020				
C. D.	0·057				
	Yield in maunds per acre				
Ethylene chlorohydrin—					
0	157	228	198	223	180
0·5 per cent	184	219	214	232	226
1·0 per cent	211	258	194	230	220
1·5 per cent	209	256	216	228	221
S. E.	±11·8				
C. D.	33·0				

TABLE II—(contd.)

The effect of seed treatment with chemicals on sprout emergence, rate index, and the yield of potatoes (Series II)—contd.

Variety	Up-to-date
Source of seed	Kufri
Date of harvest	Middle September, 1954
Date of planting	November 20, 1954
after treatment	1954-55
Year	

Treatments		Thiourea		Ammonium Thiocyanate	
	0	1 per cent	2 per cent	0·5 per cent	1·0 per cent
		Percentage Emergence, 35 days after planting			
Ethylene chlorohydrin—					
0	60·5	80·5	82·2	70·8	60·5
0·5 per cent	72·2	85·8	81·3	71·7	79·7
1·0 per cent	71·1	88·0	80·8	64·2	73·3
1·5 per cent	78·7	86·2	85·0	67·8	72·8
		Rate Index (Bartlett)			
Ethylene chlorohydrin—					
0	0·406	0·403	0·472	0·511	0·421
0·5 per cent	0·499	0·653	0·522	0·569	0·592
1·0 per cent	0·486	0·660	0·506	0·596	0·610
1·5 per cent	0·525	0·623	0·508	0·607	0·639
S. E.	±0·024	±0·024	±0·024	±0·024	±0·024
C. D.	0·068	0·068	0·068	±0·068	±0·068
		Yield in maunds per acre			
Ethylene chlorohydrin—					
0	208	187	208	209	166
0·5 per cent	220	213	182	197	206
1·0 per cent	224	211	192	180	194
1·5 per cent	241	205	207	193	203
S. E.	±14·6	±14·6	±14·6	±14·6	±14·6
C. D.	41·0	41·0	41·0	41·0	41·0

The experiments under this series confirmed the findings of series I in regard to the effectiveness of the three chemicals in stimulating emergence. The results showed that: (i) with the fully dormant seed tubers of D.R.R., 1 or 1·5 per cent ethylene chlorohydrin was more effective than the lower concentration, both on stand and the yield. A similar trend was noticed with the 'Up-to-date' variety although not significantly marked, (ii) in the case of D.R.R., 2 per cent thiourea was nearly as good as 1 per cent, in speeding up emergence and attaining good stands, but tended to be less effective than the lower concentration in regard to yield. In the case of 'Up-to-date,' thiourea at either concentration improved germination but this advantage was not reflected in yield, (iii) 0·5 per cent thiocyanate compared favourably with 1 per cent thiourea in all respects but 1 per cent thiocyanate had an adverse effect on the yield of the variety Up-to-date, (iv) the combined treatment, 1 per cent or 1·5 per cent ethylene chlorohydrin and 1 per cent thiourea again gave the maximum response in the case of 'D.R.R.' but with 'Up-to-date', none of the double treatments was any better than the control as the seed had already become partially non-dormant.

Series III

In this series, only the germination of the treated and untreated tubers was recorded. The percentage germination is given in Table III

TABLE III

The effect of seed treatment with chemicals on the sprout emergence (Series III)

Variety	Hybrid O.N. 249
Date of harvest at Patna	December 6, 1953
Date of planting after treatment	December 8, 1953

Date of observation	Nov. 26, 1953	Dec. 30, 1953	Jan. 1, 1954	Jan. 8, 1954	Jan. 12, 1954	Jan. 16, 1954	Jan. 21, 1954
O : Control	—	—	—	—	—	—	—
E : Ethylene Chlorohydrin 3 per cent	—	—	2	3	3	6	10
T : Thiourea 1 per cent	1	43	65	71	76	87	92
TE :	6	64	80	81	81	83	91
ET : (i)	—	6	28	42	44	56	70
„ (ii)	—	12	38	48	56	68	86

Germination started about 18 days after planting in seed pieces treated with 1 per cent thiourea alone (T) or in combination with ethylene chlorohydrin (TE). The combined treatment hastened the speed of emergence although thiourea alone was as good in regard to the number of plants emerged 44 days after planting. Of the two types of combined treatments, the effect was less marked when thiourea treatment followed ethylene chlorohydrin than when the treatment order was reversed. It is interesting to note that the untreated cut pieces did not sprout until towards the end of the crop season and were found intact when dug out on the 25th March 1954.

Discussion

The foregoing results confirm the findings of Denny (*loc. cit*) that treatment of seed potatoes with ethylene chlorohydrin, thiourea or ammonium thiocyanate in certain concentrations can overcome or abridge dormancy. With the varieties, 'Up-to-date' and 'D.R.R.', the seed of which is imported from the hills for planting in the plains to a considerable extent, the optimum concentration of ethylene chlorohydrin for dip treatment is indicated to be 1·5 per cent and of thiourea and ammonium thiocyanate 1 per cent and 0·5 per cent respectively for soak treatment. Higher concentrations of ethylene chlorohydrin caused only a small further improvement, while those of thiourea were without much further effect and of thiocyanate were less effective or even harmful. From the practical point of view, the thiocyanate would not appear to be suitable as its behaviour is somewhat inconsistent, there being a narrow range between the effective and the toxic concentrations. Ethylene chlorohydrin would not be safe in the hands of ordinary growers, as the vapours of this chemical are toxic to human beings and animals. Soak treatment in thiourea (1 per cent) for one hour is a simple, safe and successful measure that could be recommended for adoption by the growers. Moreover, as this chemical causes multiple buds to sprout from the same eye, it is of special interest to the seed grower. Skvarnikov and Solomko [1954] in Russia also considered thiourea to be the most effective for stimulating the sprouting of dormant seed.

It must be emphasised that treatment effects were generally better with 'D.R.R.' which has a longer period of dormancy than with 'Up-to-date' which has only a short period of dormancy. Further, the magnitude of the effects is more if the seed is treated soon after the harvest than later when the seed has already lost part of its dormancy.

Practical Application

With a view to test the efficacy of the successful treatments on a large scale, dormant seed of D.R.R. was obtained from Kufri in 1954-55, and a part of it was planted at Patna after treatment with thiourea alone and the rest after combined treatment with ethylene chlorohydrin and thiourea, on an area of about 6·5 acres in the second and third weeks of November. Germination started in about a fort-

night after planting and was rapidly completed in the case of the double treatment, while in the case of the single treatment the tubers started to germinate a little later and the germination was gradually completed. At harvest, the yields from these plots averaged over 200 md. per acre, which is a fairly high yield for a late crop. In 1955-56, about 5 acres were planted at Patna with the treated seed with similar results. Now, seed treatment has become a standard practice in the Central Potato Research Institute for breaking dormancy and treatment with thiourea (1 per cent) is recommended to cultivators for the purpose as a safe, simple and cheap method.

The tubers are washed, air-dried and cut into pieces containing 1—2 eyes. The seed pieces are then soaked in a 1 per cent aqueous solution of thiourea for one hour. Thereafter the solution is drained into another container for re-use. Repeated use of the solution for 7-8 times does not impair its potency. The seed pieces are ready for planting immediately after treatment, but if planting is delayed, the seed pieces should be air-dried in shade, as wet tubers if heaped up are prone to rottage.

The treatment is quite cheap and is within the reach of an ordinary Indian cultivator. One pound of thiourea costing about Rs. 5 is sufficient to treat about 10 md. of seed potatoes. The cost of treatment is negligible compared to the high returns that follow.

Summary

Multi-factor experiments were conducted at the Central Potato Research Institute, Patna, to study the effect of dormancy-breaking chemicals on freshly-harvested potatoes obtained from the hills, in regard to percentage emergence, rate index and yield, with a view to formulate a simple method suitable for adoption by potato growers in India. Two commercial varieties, D.R.R. and Up-to-date, the seed of which is generally obtained from the hills for planting in the plains, were included in these experiments. The treatments comprised all combinations of (*a*) dip treatment of cut pieces in ethylene chlorohydrin at varying concentrations and (*b*) soak treatment in thiourea and ammonium thiocyanate at different concentrations.

An additional experiment with seed freshly-harvested at Patna was also laid out to study the effect of 'dip' treatment in ethylene chlorohydrin and 'soak' treatment in thiourea, singly and in combination, on sprout emergence.

The results showed that : (1) in the case of 'D.R.R.' variety which has a long rest period, 1 to 1·5 per cent ethylene chlorohydrin, 1 per cent thiourea or 0·5 per cent to 1 per cent ammonium thiocyanate can effectively break dormancy and increase the cropping value of the dormant seed. Higher concentrations (3 or 4·5 per cent) of ethylene chlorohydrin were only slightly better than 1·5 per cent concentration. Higher concentration (2 per cent) of thiourea was not better than its lower dose and that of the thiocyanate had hardly any effect, (2) in the case of the variety, 'Up-to-date', which has a short rest period of about two months' duration, the treatment of seed with any of the three chemicals towards the end of dormancy period, improved the stand and speed of germination to a small extent. The yield was influenced

favourably by 1·5 per cent ethylene chlorohydrin, (3) the combined treatment of thiourea 1 per cent and ethylene chlorohydrin 1—1·5 per cent, accelerated the speed of germination and the yield of the fully dormant tubers, (4) in the case of the variety O.N. 249, it was found that soak treatment of cut seed pieces of freshly-harvested tubers in 1 per cent thiourea alone or in combination with dip treatment in 3 per cent ethylene chlorohydrin induced prompt sprouting. The combined treatment of thiourea followed by ethylene chlorohydrin was, however, more effective than that of ethylene chlorohydrin followed by thiourea.

It is concluded that soak treatment of cut seed pieces with 1 per cent aqueous solution of thiourea for one hour, before planting, is a simple, safe, successful and cheap method for breaking dormancy of freshly-harvested tubers, which can be adopted on a large-scale by potato grower.

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AN IMPROVED METHOD FOR DIRECT ESTIMATION OF FREE TOCOPHEROL IN SMALL QUANTITIES OF OILSEEDS

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TOCOPHEROL (Vitamin E or antisterility vitamin) is an essential factor in the diet of men and animals and the requirements of this vitamin are met mainly from oils and fats ingested in the food. Breeding of varieties of oilseeds of higher tocopherol content is hence a matter of importance for the future. Since tocopherol acts as fat anti-oxidants this will result in edible oils and fats of improved keeping qualities as well.

The accurate determination of tocopherol in oils offers considerable difficulties even when large specimens are available, and time and expense are no consideration; the problem is more difficult when determinations have to be done inexpensively and with small seed specimens as required in plant breeding. Methods involving saponification of fat and extraction of unsaponifiable matter are known to be liable to error due to incomplete extraction of tocopherol from aqueous alcholic solutions of soaps and to the tendency for tocopherol to get oxidised in alkaline soap solutions [Moss and Drummond, 1938 ; Parker and McFarlane, 1940 ; Devlin and Matill, 1942]. To eliminate these sources of error, Parker and McFarlane [1940] suggested the direct estimation of tocopherols in oils by the Emmerie and Engel reaction. This will indicate only the free tocopherol content but the amount of esterified tocopherol in vegetable oils and fats is generally considered to be quite small [Moss and Drummond, 1938].

Kaunitz and Beaver [1944] reported that when estimation of tocopherol by the Emmerie and Engel method is conducted in the presence of fats, a correction has to be made for the inhibition or depression of colour produced by the fat. The nature of inhibition is as follows : suppose $X\mu$ of tocopherol in a fixed volume of reaction solution develops an extinction coefficient (E.C.) A when treated with the Emmerie and Engel reagent, in the absence of any fat. When the treatment is done in the presence of Y mg. of a fat, all other conditions remaining identical, it is found that the E.C. developed A is lower than A. The difference between A and A′ is due to the inhibiting effect of fat. The ratio A/A′ may be termed the inhibition coefficient (I.C.) at the particular weight of fat used. It has been suggested that this inhibition may perhaps be due to presence of unsaturated substances [Kaunitz and Beaver, 1944 ; Stern and Baxter, 1947] but no further investigations about its nature and mechanism has yet been reported.

The importance of this inhibitive effect depends on the fact that a large and variable correction based on the I.C. of the fat at the weight taken, has to be made to arrive at the actual proportions of tocopherol in the fat. Percentage transmissions can be measured only to about 1 per cent and the error due to this will be of

the order of about 20—25 per cent in the case of fats of 0·05 per cent tocopherol content when 100 mg. are used for the determinations and I.C. is 6 at 100 mg. concentration [Kaunitz and Beaver, 1944] and would be higher as tocopherol content or weight of fat decreases. If inhibition can be eliminated or limited to a small minimum value, then the direct determination of tocopherol content of oils will be accurate, simple and inexpensive.

Kaunitz and Beaver [1944] used unrefined sesame and other oils for their experiments. Hence, apart from non-glyceridic impurities, small amounts of saturated and unsaturated acids will be present in addition to the glycerides. The nature and proportions of non-glyceridic substances vary in different fats but these are usually present in such small proportions as not to cause much error, except when highly unsaturated substances like carotene or vitamin A is present, and this is rarely the case in vegetable oils and fats. Inhibition produced by the different fatty acid derivative (F.A.D.) has hence been studied first.

The inhibition as reported in the literature is observed when a known amount of tocopherol and fat is dissolved in purified petroleum ether and to this mixture a measured amount of ferric chloride-dipyridyl mixture either in purified absolute ethanol or acetic acid, is added, made up to volume and transmission measured, using M 515 filter; or alternatively when to tocopherol-fat solution in petroleum ether a known amount of dipyridyl solution is added [Stern and Baxter, 1947], and mixed, followed by ferric chloride, the rest of the procedure being as before.

To eliminate interference by substances which can be oxidised by ferric chloride the various F.A.D. were carefully purified and in the case of unsaturated F.A.D. were submitted to final purification by treatment with excess ferric chloride. The effect of the presence of varying quantities of these F.A.D. on the E.C. developed when known quantities of tocopherol were treated with the Emmerie and Engel reagent, were then studied. The different F.A.D. prepared and the methods of purification used were as follows:

(1) *Saturated acids.* One specimen was obtained by exhaustive acetone-permanganate oxidation of fat from *Myristica malabarica* which contains about 85 per cent saturated acids, isolating the fully saturated glycerides and hydrolysing these. Another specimen was prepared by crystallising C.P. stearic acid three times from ethyl alcohol.

(2) *Ethyl stearate.* Pure stearic acid obtained by repeated crystallisation of C. P. acid from alcohol was esterified with absolute ethanol and the ethyl stearate purified by distillation in vacuum.

(3) *Fully saturated glycerides.* This was prepared by exhaustive acetone-permanganate oxidation of coconut oil and removing the acidic products with potassium carbonate and finally with potassium hydroxide solutions. The neutral product thus obtained was submitted to a second oxidation to complete the destruction of unsaturated impurities.

(4) *Unsaturated acids.* Mixed fatty acids from groundnut oil were submitted to Twitchell's lead salt separation, liquid acids isolated, dissolved in 2 per cent aqueous potassium hydroxide in presence of alcohol and solution extracted three times with sulphuric ether to remove unsaponifiables. The unsaturated acids were recovered and submitted to a final purification with ferric chloride as follows : 5 gm. of the acid were dissolved in 25 cc. of purified alcohol or sulphuric ether, 1 gm. ferric chloride dissolved in 10-15 cc. of alcohol added and the mixture shaken well for 30 minutes. This was then diluted with water, extracted with ether and the last traces of ferric chloride washed off with dilute hydrochloric acid followed by water. The ethereal extract was shaken with a little activated charcoal for 10 minutes, decanted, dried over anhydrous sodium sulphate, filtered and solvent removed.

(5) *Ethyl oleate.* C. P. oleic acid (Merck) was esterified with ethyl alcohol, the ethyl oleate fractionated in a vacuum, the middle portions of the distillate collected and further purified by treatment with ferric chloride as described for unsaturated acids.

(6) *Purified groundnut oil.* Groundnut oil was deacidified by dissolving in purified ether and washing with 0·5 per cent potassium hydroxide solution containing a little ethyl alcohol till all acidic matter was removed ; the ethereal solution was then washed free of alkali and the neutral oil isolated. This was purified by treatment with excess of ferric chloride as described for unsaturated acids.

Reactions were done in purified ethyl alcohol in case of saturated acids, ethyl oleate, ethyl stearate, and unsaturated acids. For fully saturated glycerides and groundut oil a solvent mixture containing 33 per cent purified alcohol and 66 per cent benzene was used. Since impurities are found in some specimens of Analar reagent which interfere with the colour, the benzene has to be carefully purified. For this Analar benzene was repeatedly shaken with concentrated sulphuric acid till only a very faint yellow colour was produced, washed with alkali, dried and distilled. This was mixed with half its volume of purified ethanol and kept over ferric chloride for 24 hours and then distilled. The distillate (500 cc.) was treated with 5 gm. of finely powdered ferrous sulphate and 5 cc. concentrated sulfuric acid, kept at room tempe rature for 24 hours, then refluxed for three hours, and distilled. The mixture can be dehydrated when necessary by treatment with lime and redistillation. The solvent consists of the binary azeotrope of benzene with ethyl alcohol, b.p.68°C. In all cases the tocopherol, dipyridyl, and ferric chloride were dissolved in purified ethyl alcohol and requisite volumes pipetted out.

The effect of different F.A.D. on the estimation of tocopherol [Kaunitz and Beaver, 1944] was studied as follows : Requisite volumes of tocopherol and F.A.D. solutions were pipetted out into a 10 cc. graduated cylinder provided with a ground glass stopper and the necessary solvent or solvent-mixture added till a volume of 6-7 cc. was reached. 0·5 cc. of a 0·5 per cent solution of dipyridyl was then added and mixed, followed by 0·4 cc. of a 0·6 per cent solution of ferric chloride. The volume was made up to 10 cc., mixture shaken well and allowed to develop colour in the dark. The percentage transmission was measured at intervals of 15 minutes using a 1 cm. cell and M 515 filter. The percentage transmission without F.A.D.

is the reading given by a specific weight of tocopherol against the pure reagent blanks while the percentage transmission with F.A.D. gives the reading by the same weight of tocopherol in the presence of a particular weight of F.A.D. against a blank consisting of the reagents added to the same amount of F.A.D. as in the experimentals.

The results obtained with saturated fatty acids, ethyl stearate, and fully saturated glycerides are given in Table I. These do not produce any inhibition under the experimental conditions. The saturated paraffin chain, free carboxylic group or carboxylic group esterified by aliphatic alcohol or glycerol are hence not responsible for the inhibition.

TABLE I

Effect of saturated F.A.D. on the Emmerie and Engel Reaction

Serial No.	Nature of F.A.D.	Wt. of F.A.D. mg. approx.	Tocopherol content approx. μ	Percentage transmission (Cell 1 cm.: Filter M 515)		
				Without F.A.D.	With F. A. D.	
					15 min.	60 min.
1	Sat. Acid from *M. malabarica*	100	35	75	76	76
		25	35	75	75	75
		100	69	56	56	57
		25	69	56	56	57
	Ethyl stearate	100	35	75	75	75
		25	35	75	75	75
		100	69	56	57	56
		25	69	56	56	56
		100	106	40	41	41
		25	106	40	41	40
3	Fully saturated glycerides from coconut oil	100	33	76	75	76
		50	36	74	74	73
		100	69	56	55	56
		50	82	49	50	50
		100	108	39	41	40
		50	..	32	32	32

The results obtained with unsaturated fatty acids, ethyl oleate, and purified groundnut oil are given in Table II and show considerable inhibition when the weight of F.A.D. taken is high enough. Since saturated paraffin chain or free or esterified carboxylic group do not produce inhibition, inhibition must be due to the carbon-carbon double bonds in the unsaturated F.A.D.

TABLE II

Effect of unsaturated F.A.D. on Emmerie and Engel Reaction

Serial No.	Nature of F.A.D.	Wt. of F.A.D. mg. approx.	Tocopherol approx. μ	Percentage transmission (Cell 1 cm.: Filter M 515)		
				Without F.A.D.	With F.A.D.	
					15 min.	60 min.
1	Unsaturated acids from groundnut oil.	100	36	74	81	81
		50	36	74	80	80
		25	36	74	81	80
		5	36	74	74	74
		100	69	56	67	69
		50	69	56	68	68
		25	69	56	64	63
		5	69	56	56	56
		100	108	39	56	55
2	Ethyl oleate	100	33	76	81	79
		25	33	76	78	77
		15	33	76	76	76
		100	69	56	66	65
		25	69	56	59	57
		15	69	56	57	57
		5	69	56	56	57
		100	106	40	57	57
		50	106	40	53	51
		25	106	40	53	50
		15	106	40	42	41
		5	106	40	41	40

TABLE II—(contd.)

Effect of unsaturated F. A. D. on Emmerie and Engel Reaction

Serial No.	Nature of F.A.D.	Wt. of F.A.D. mg. approx.	Tocopherol approx. μ	Percentage transmission (Cell 1 cm. : Filter M 515)		
				Without F.A.D.	With F.A.D.	
					15 min.	16 min.
3	Purified groundnut oil	100	66	57	69	70
		50	66	57	63	62
		25	66	57	59	59
		10	66	57	57	57
		100	104	41	53	52
		50	104	41	47	46
		25	104	41	47	45
		10	104	41	41	41

Results in Table II also show that the amount of inhibition is dependent on the nature of the unsaturated F.A.D. and the amount of the latter present ; it is, however, by no means proportional to the amount of F.A.D. present. In the experiments reported, when the weight of unsaturated F.A.D. was 10 mg. or less, practically no inhibition was observed and this perhaps explains Stern and Baxter's report [1947] that little or no inhibition was produced when a sesame oil concentrate containing 27 per cent tocopherol was analysed by the Kaunitz and Beaver method [1944].

Since inhibition is thus traceable to the unsaturation in the fatty acid molecules it can be produced by other unsaturated substances as well and when the method is used for the determination of tocopherol in the presence of unsaturated substances correction has to be made for this source of error.

The above inhibition produced by unsaturated fatty acids and their derivatives can be due to two reasons. The tocopherol may combine with the unsaturated F.A.D. to form a molecular complex which might be more resistant to oxidation by ferric chloride, whence incomplete oxidation of tocopherol may be taking place. This explanation is not probable due to several reasons.

(1) Absence of inhibition with saturated fatty acids or esters shows that combination does not take place with free or esterified carboxylic groups or saturated paraffin chains. There is little evidence that tocopherol can combine with unsaturated substances at carbon-carbon double bonds.

(2) When less than about 10 mg. of unsaturated F.A.D. are used as in Table II there is practically no inhibition in spite of the fact that even at this concentration the unsaturated F.A.D. is present at the ratio of about 700-800 mols. per mol. of tocopherol when about 30μ of tocopherol is used. Hence any chemical affinity between the two must be so small that the complex will decompose completely on diminishing the concentration of tocopherol as happens when it gets oxidised by ferric chloride.

(3) If inhibition is due to formation of molecular complexes then if a definite weight of tocopherol is oxidised with ferric chloride in the absence of F.A.D. and then a known weight of F.A.D. is added, there will not be any lowering of the E.C. and hence no inhibition. This has been studied as follows :

A known amount of tocopherol was pipetted into a 10 cc. graduated cylinder with a ground glass stopper ; 5 cc. of purified ethyl alcohol or alcohol-benzene added and oxidised by adding first dipyridyl solution and then ferric chloride. The reaction was allowed to go in the dark for five minutes ; by this time the oxidation was complete with pure tocopherol solutions [Stern and Baxter, 1947]. The E.C. at this stage was obtained by making up one of the replicates to 10 cc. and finding out the percentage transmission. To another replicate a known amount of unsaturated F.A.D. was added, mixed and made up to volume. The E.C. of the samples with and without F.A.D. were measured at 5 minutes intervals. Samples without F.A.D. showed no decrease or increase in percentage transmission. Samples with added F.A.D. showed progressively increasing percentage transmission as shown in Table III. It will be observed from Table II that when ferric chloride is added to a mixture of purified unsaturated F.A.D., tocopherol and dipyridyl, a constant E.C. is reached within 15 minutes which does not alter much later (one hour). As will be explained later, the inhibition shown by a particular weight of an F.A.D. can show different values after different treatments and when the inhibition produced in the normal way [Kaunitz and Beaver, 1944] is measured simultaneously, it is found that the increase in percentage transmission observed on adding a known weight of F.A.D. to the completed Emmerie and Engel reaction almost comes to a stop at some point below the percentage transmission observed in the normal procedure after 15 minutes. A comparatives tudy of the results in Tables II and III shows further that stable equilibrium is reached sooner when the ferrous chloride is produced in the presence of dipyridyl and unsaturated F.A.D. than when the unsaturated F.A.D. is added to ferrous chloride-dipyridyl complex : perhaps the latter is more resistant towards oxidation by fat peroxides than the ferrous chloride itself ; this will be discussed again later.

The results in Table III show that building up of more stable molecular complex is not the reason for inhibition and that this can be explained only by assuming complete oxidation of tocopherol followed by oxidation of a part of the ferrous iron formed back to ferric iron either by the unsaturated F.A.D. or some reaction product of this simultaneously present, for careful purification of the samples leads us to

believe that little or no reactive non-fatty matter will remain behind. That inhibition is actually due to oxidation of the ferrous chloride to ferric chloride is established by the experiment recorded in Table III wherein the percentage transmission

TABLE III

Effect of adding unsaturated F.A.D. on completed Emmerie and Engel Reaction

Sl. No.	Nature of F.A.D.	Wt. of F.A.D. mg. approx.	Tocopherol approx. μ	Percentage transmission (Cell 1 cm. : Filter M 515)			
				Without F.A.D.	With F.A.D.		
					15 min.	30 min.	45 min.
1	Unsaturated acids from groundnut oil	100	64	58	62	67	69
		100	102	42	48	55	62
		50	64	58	59	60	62
		50	102	42	46	48	56
		25	64	58	57	58	58
		25	102	42	42	43	44
2	Ethyl Oleate	100	64	58	59	60	61
		100	102	42	43	44	46
		50	64	58	58	58	59
		50	102	42	42	43	44
3	Groundnut oil purified	100	64	58	71	75	78
		100	102	42	67	72	78
		50	64	58	70	74	80
		50	102	42	60	66	75
		25	35	75	85	86	88
		25	66	57	75	77	78
		25	104	41	68	69	70

increases progressively after addition of the unsaturated F.A.D. and hence the final explanation may be that fat peroxides present in the unsaturated F.A.D. oxidise part of the ferrous chloride formed during oxidation of tocopherol. It has been known for some time that peroxides occur in unsaturated hydrocarbon oils

and also, in unsaturated F.A.D. under ordinary conditions of storage, which can oxidise ferrous iron to ferric iron and methods have been described to determine the peroxide content of these substances by reacting ferrous ammonium sulfate with weighed quantities of the materials in the presence of ammonium thiocyanate and measuring the amount of ferric thiocyanate formed [Young, Vogl and Nieuwland, 1936 ; Yule and Wilson, 1931]. Estimation of peroxide content by this method, however, gave lower results than by acetic acid-potassium iodide method as ferrous : sulfate is less readily oxidised by peroxides than potassium iodide-acetic-acid ; similarly oxidation of ferrous chloride by unsaturated F.A.D. at room temperature may be only partial, but any way enough oxidation takes place when the amount of ferrous chloride and peroxide value of F.A.D. are large enough for distinct inhibition to be observed.

The conclusion that inhibition is produced by the weak oxidising action of fat peroxides on the ferrous chloride is further supported by the following :

(1) It is well known that unsaturated F.A.D. increases in peroxide value on standing exposed to air either as such or in solution, at comparatively low temperatures of say 20-100°C. Hence the extent of inhibition should increase under conditions when the peroxide content of the F.A.D. increases.

Differences in inhibitive power as various unsaturated fatty acid derivatives are allowed to peroxidise either as such or in solution at room temperature are given in Table IV. The experimental samples in the different instances were all derived from the same stock samples and hence the different stages of peroxidation at which the samples were examined are mentioned as stage 1, stage 2, etc. progressively. The results show that the same unsaturated F.A.D. specimen shows varying inhibition.

TABLE IV

Differences in inhibitive power of unsaturated F.A.D. at different stages of aging

Serial No.	Nature of F.A.D.	Stage	Wt. of F.A.D. mg.	Tocopherol μ	Percentage transmission (Cell 1 cm. : Filter M 515)	
					Without F.A.D.	With F.A.D.
1	Unsaturated acid from groundnut oil.	1	100	69	56	66
			50	69	56	68
			25	69	56	64
		2	25	35	75	93
			25	66	57	87
			25	104	41	84
		3	25	35	75	97
			25	66	57	95
			25	104	41	95

TABLE IV—(contd.)

Differences in inhibitive power of unsaturated F. A. D. at different stages of aging

Seral No.	Nature of F.A.D.	Stage	Wt. of F.A.D. mg.	Tocopherol μ	Precentage transmission (Cell 1 cm.: Filter M 515)	
					Without F.A.D.	With F.A.D.
2	Ethyl oleate	1	100	33	76	81
			25	33	76	78
			100	69	56	66
			25	69	56	59
			100	106	40	57
			25	106	40	51
		2	100	35	75	80
			100	72	55	72
			100	98	44	58
		3	100	35	75	95
			100	72	55	92
			100	98	44	90
			25	35	75	91
			25	72	55	85
			25	98	44	77
3	Groundnut oil	1	100	66	57	68
			100	104	41	51
			50	66	57	62
			50	104	41	47
		2	100	63	58	88
			100	101	42	86
		3	100	35	75	93
			100	104	41	78
			100	66	57	84
		4	100	35	75	99
			100	66	57	96
			100	104	41	64
		5	100	66	57	96
			100	101	41	95
			50	66	57	98
			50	101	41	95

powers depending on the state of peroxidation. Hence whenever the inhibition coefficient is to be used for calculating the tocopherol content it should be determined at the time of the estimation.

In Tables V-A and V-B. the effect of heating on the inhibitive power of different F.A.D. is given. Whereas when allowed to peroxidise at room temperature all unsaturated F.A.D. show a progressive increase in I.C.; when heated at 100°C a difference is noted between unsaturated fatty acids on the one hand and ethyl oleate and groundnut oil on the other. The unsaturated acid peroxides appear to be unstable at 100°C and decompose gradually (initial peroxide value (P.V.) of 125) (all peroxide values given in this paper are millimoles peroxide per Kg. of fat) and their inhibitive power also declines till at the end of about 5-6 hours the I.C. reaches values close to unity, P. V. at this stage is only about 10-12 and establishes directly the relation between P.V. and inhibition. In the case of ethyl oleate and groundnut oil, however, it is seen that the inhibitive power shows an increase when the specimens are heated at 100°C in presence of air and this corresponds to the increase in P.V. shown by these when heated in presence of air at 100°C as reported in the literature.

(2) If inhibition produced by unsaturated F.A.D. is due to oxidation of the ferrous chloride to ferric chloride by peroxides, treatment of the F.A.D., with ferrous sulfate should show a sharp decrease in inhibitive power also. The peroxides may not be completely decomposed by ferrous sulfate (*loc. cit.*) but a good amount of decomposition will, nevertheless, take place and a substantial reduction in inhibition after treatment will be enough to confirm the conclusion.

Five gram. of ethyl oleate (P.V. about 110) or groundnut oil (P.V. about 90) was mixed with about 40 cc. of purified ethyl alcohol, 10 cc. of a saturated aqueous solution of ferrous sulfate was added and the mixture refluxed for 1 hour. The reaction mixture was then diluted with water, extracted with ether and extract washed with dilute sulfuric acid and then with water and the F.A.D. isolated as

TABLE V-A

Effect of heating at 95-98°C on the inhibitive power of unsaturated F.A.D.

Sl. No.	Nature of F.A.D.	Wt. of F.A.D. mg.	Tocopherol μ	Percentage transmission (Cell 1 cm. : Filter M 515)	
				Without F.A.D.	With F.A.D.
1	Groundnut oil unsaturated acids				
	(*a*) Before heating (Peroxide value 125).	50	69	56	91
		50	104	42	84
		25	69	56	82
		25	104	42	76
	(*b*) After 3 hrs. heating	50	69	56	72
		50	104	42	57
		25	69	56	66
		25	104	42	54

TABLE V-A—(contd.)

Effect of heating at 95-98°C on the inhibitive power of unsaturated F. A. D.

Sl. No.	Nature of F.A.D.	Wt. of F.A.D. mg.	Tocopherol μ	Percentage transmission (Cell 1 cm. : Filter M 515)	
				Without F.A.D.	With F.A.D.
	(*c*) After 5 hrs. heating (Peroxide value 12-13)	50	69	56	61
		50	104	42	47
		25	69	56	60
		25	104	42	47
2	Ethyl oleate				
	(*a*) Before heating	50	69	56	80
		50	104	41	73
		25	69	56	75
		25	104	41	61
	(*b*) After 4 hrs. heating	50	69	56	82
		50	104	41	77
		25	69	56	79
		25	104	41	68

usual. They show P.V. of the order of 1-4 only. A weighed amount of this treated F.A.D. was then dissolved in the proper solvent or solvent-mixture and its inhibitive power measured as usual. The inhibitive power of the untreated F.A.D. using the same weight as in the former case, was also measured at the same time. The results (Table VI) show substantial reduction in inhibitive power on treatment with ferrous sulfate. This confirms further that inhibition is caused by the oxidising action of fat peroxides on the ferrous chloride.

TABLE VI

Effect of treatment with ferrous sulfate solution on the inhibitive power of unsaturated F.A.D.

Sl. No.	Nature of F.A.D.	Wt. of F.A.D. mg.	Tocopherol μ	Percentage transmission (Cell 1 cm. : Filter M 515)	
				Without F.A.D.	With F.A.D.
1	Ethyl oleate				
	(*a*) Before treatment	100	35	75	95
		100	72	55	92
		100	98	44	90
		25	35	75	91
		25	72	55	85
		25	98	44	77

TABLE VI—(contd.)

Effect of treatment with ferrous sulfate solution on the inhibitive power of unsaturated F.A.D.

Sl. No.	Nature of F.A.D.	Wt. of F.A.D. gm.	Tocopherol μ	Percentage transmission (Cell 1 cm. : Filter M 515)	
				Without F.A.D.	With F.A.D.
	(*b*) Immediately after treatment	100	35	75	78
		100	72	55	60
		100	98	44	47
		25	35	75	77
		25	72	55	58
		25	98	44	45
	(*c*) After keeping exposed to air for 24 hrs. after treatment (Room temp.)	100	35	75	81
		100	72	55	72
		100	98	44	58
2	Groundnut oil— (*a*) Before treatment	100	69	56	82
		100	102	42	70
		25	69	56	67
		25	102	42	54
	(*b*) After treatment	100	74	54	62
		100	100	43	51
		25	74	54	60
		25	100	43	49

It is well known that peroxides in fatty acid derivatives can be completely destroyed by refluxing with alcoholic potash : the hydrolysed products with all peroxides destroyed can be isolated by treatment with mineral acid and extraction with ether. Samples of groundnut oil (P.V. 200), ethyl oleate (P.V. 120), and oleic acid (P.V. 95) which showed high inhibition were treated with excess alcoholic potash for 2 hours on the water-bath and hydrolysed products recovered, working

at as low temperatures as possible. Immediately after isolation they showed P.V. of the order of 0-1 only and at this stage showed practically no inhibition. Exposure to air for a few hours at room temperature developed appreciable P.V. and inhibitive effect also simultaneously increased.

Treatment of high peroxide value fats or fatty acid derivatives with 85 per cent sulfuric acid as recommended by Parker and McFarlane (1940) or by acetic acid-potassium iodide solution also yielded similar results, the P. V. of the treated products coming down to 0-2 from initial values as high as 200—300 or higher and simultaneously with the destruction of the peroxides the inhibitive effect was also reduced to almost near zero values when the freshly recovered products were examined. This indicates that it is the peroxides which oxidise ferrous sulfate, or acetic acid-potassium iodide and which are decomposed almost completely by treatment with alcoholic potash, or 85 per cent sulphuric acid which produce inhibition.

There is hence little significance in assigning any I. C. to any oil since this will vary with the degree of autoxidation it has undergone or its peroxide value.

Apart from the fact that inhibition is caused by peroxides some other features also have come to light during these investigations. The reaction between peroxides and ferrous chloride appears to reach an equilibrium under definite experimental conditions, and under these approximately the same relative proportion of ferrous chloride is oxidised irrespective of the actual amount of ferrous chloride present. The relative proportions oxidised depend only on the amount of fat present and the degree of autoxidation of fat or its P.V. The last column in Table V-B relating to autoxidation of groundnut oil shows this clearly and the ratio of E.C. of (*b*)/E.C. of (*a*) $\times 100$ is seen to remain constant approximately for the same weight of fat at different concentrations of tocopherol. It is this equilibrium which forms the basis of the Kaunitz and Beaver effect and it holds more or less accurately when the inhibition coefficient developed in presence of fat is not less than half that developed in its absence.

It has also been brought to light during these studies that in presence of peroxidised fat, the percentage transmission developed during the first hour or so depends not only on the amount of fat and its P.V. but also on the order of addition of the reagents : this does not affect the results in the absence of fat. In presence of peroxidised fat the highest E.C. is obtained when ferric chloride is added to a mixture of tocopherol, fat and dipyridyl solution, the reading obtained by this procedure, however, remains steady over any period of time (in the darkness) only when the P.V. of fat is low ; in the case of fat of higher P.V. the percentage transmission which reaches a minimum in the first 5-15 minutes gradually increases. Much lower values are obtained for the E.Cs. when the order of addition of reagents is changed and dipyridyl is added to a mixture of fat, tocopherol and ferric chloride solution 3 to 4 minutes after mixing. The value obtained by this procedure remains steady over long periods showing that a stable equilibrium has been attained. The difference in percentage transmission obtained by the two procedures for the same

TABLE V-B

Effect of heating at 95-98°C on the inhibitive power of unsaturated F. A. D.

Serial No.	Nature of F.A.D.	Wt. of F.A.D. mg.	Tocopherol μ	Without F.A.D. (*a*)	Percentage transmission (Cell 1cm.: Filter M 515)	
					With F.A.D. (*b*)	E.C. of (b) / E.C. of (a) × 100
	Groundnut oil :					
1	(*a*) Before incubation P.V. of specimen, about 15	50	69	56	66	72
		50	104	41	52	73
		25	69	56	60	88
		25	104	41	46	87
	(*b*) After 9 hrs. incubation P.V. of specimen 22	50	69	56	68	67
		50	104	41	55	67
		25	69	56	65	74
		25	104	41	51	76
	(*c*) After 16 hrs. incubation P.V. of specimen 30	50	75	54	74	49
		50	102	42	64	51
		25	75	54	69	60
		25	102	42	53	72
	(*d*) After 19 hrs. incubation P.V. of specimen 40	50	75	54	78	40
		50	102	42	69	43
		25	75	54	72	53
		25	102	42	59	61
	(*e*) After 25 hrs. incubation P. V. of specimen 65	50	77	53	92	13
		50	111	38	90	11
		25	77	53	93	11
		25	111	38	88	13

amounts of F.A.D. and tocopherol and some F.A.D. samples showing high inhibitive power are given in Table VII, by way of illustration. They have no absolute significance since the inhibitive power of any sample of F.A.D. is variable. In view of this observation, it is evident that if during reaction by the technique of adding ferric chloride to the mixture of fat, tocopherol and dipyridyl (ferric chloride last technique) any local concentration of ferrous chloride should develop, then the E.C. developed will be lower than that developed if local concentrations are entirely avoided ; this will create very poor reproducibility of results when the inhibitive power of fat is high. Further for the same substance, when the degree of inhibition by the ferric chloride last technique increases (or decreases) a similar increase (or decrease) takes place when the colour is developed by the dipyridyl last technique as well and in the case of various F:A.D. subjected to heating at 97-98°C, the results by both techniques are given in Table VIII.

The explanation for the difference in E.C. by the ferric chloride last and dipyridyl last techniques may be as follows :

The amounts of different reactants and their concentrations are the same in both the cases. In the dipyridyl last technique, the fat peroxides can act on the ferrous chloride directly, and since oxidation of tocopherol by ferric chloride is finished in less than 2 minutes the fat peroxides get some time to act on the ferrou. chloride and convert this partially to ferric chloride, and this appears to be a comparatively slow reaction. The combination between dipyridyl and ferrous chloride is on the other hand instantaneous and a good proportion of ferrous chloride produced during oxidation is immediately converted to this in the ferric chloride last technique. The fat peroxides appear to act more slowly on this ferrous chloride-dipyridyl complex than on the free ferrous chloride (see Table III). Thus the difference in E.C.s obtained by ferric chloride last and dipyridyl last technique is due to two reasons :

(1) dipyridyl-ferrous chloride complex is more stable towards oxidation by fat peroxides than ferrous chloride and

(2) formation of the dipyridyl-ferrous chloride complex is almost instantaneous whereas reaction between ferrous chloride and fat peroxide is appreciably slower.

The foregoing results indicated the possibility that if fats are examined for tocopherol content when as fresh as possible, then the value of the I.C. will be minimum. But before studying this it appeared desirable to see whether water and dilute alkali soluble impurities in crude fats or the glycerides themselves, could in any other way interfere with the determination. The crude oils will contain various amounts of free fatty acids and other extractives as well, and hence the effect of purified F.A.D. (*loc. cit.*) on the Emmerie and Engel reagent was studied. For this 0·5 cc. of 0·5 per cent dipyridyl and 0·3 cc. of 0·6 per cent ferric chloride solution were added to 3 cc. of alcohol or alcohol-benzene mixture containing the respective fatty acid or derivative in the required quantity and the reading was taken 15 and

TABLE VII

Difference in reading for $FeCl_3$ last and Dipyridyl last techniques for inhibition caused by unsaturated F.A.D.

(Cell 1 cm. : Filter M 515)

Serial No.	Nature of F.A.D.	Wt. of F.A.D. mg.	'$FeCl_3$ last' percentage transmission		'Dipyridyl last' percentage transmission	
			Without F.A.D.	With F.A.D.	Without F.A.D.	With F.A.D.
1	Unsaturated acids from groundnut oil (purified)	100	56	67	61	95
		100	39	56	42	95
		50	74	80	61	95
		50	56	68	42	96
		25	74	81	61	98
		25	56	64	42	97
2	Ethyl oleate	100	56	66	56	99
		100	40	57	40	99
		50	56	64	61	97
		50	40	53	43	98
		25	56	59	..	..
		25	40	51	..	..
		10	..	..	76	100
		10	..	..	56	100
		10	..	..	40	97
3	Groundnut oil (purified)	100	57	69	57	95
		100	41	69	57	90
		50	57	63	57	94
		50	41	47	40	90
		25	57	59	..	..
		25	41	46	..	..
		100	..	..	76	99
		50	..	..	76	100

TABLE VIII

Variation in percentage transmission by different techniques, when unsaturated F.A.D. are progressively heated at 98°C

(Cell 1 cm. : Filter M 515)

Serial No.	Nature of F.A.D.	Wt. of F.A.D. mg.	Percentage transmission without F.A.D.	Percentage transmission with F.A.D.	
				$FeCl_3$ last technique	Dipyridyl last technique
1	Groundnut oil unsaturated acids : (*a*) Before heating	25	56	32	95
		25	42	76	94
		50	56	91	98
		50	42	84	97
	(*b*) After 3 hrs. heating	25	56	66	79
		25	42	54	66
		50	56	72	87
		50	42	57	83
	(*c*) After 5 hrs. heating	25	56	60	68
		25	42	45	55
		50	42	61	71
		50	42	47	63
2	Ethyl oleate, old specimen : (*a*) Before heating	25	56	75	96
		25	41	61	94
		50	56	80	98
		50	41	73	98
	(*b*) After 4 hrs. heating	25	56	79	98
		25	41	68	96
		50	56	86	89
		50	41	77	98

TABLE VIII—(contd).

Variation in percentage transmission by different techniques, when unsaturated F. A. D. are progressively heated at 98°C.

(Cell 1 cm. : Filter M 515)

Serial No.	Nature of F. A. D.	Wt. of F.A.D. mg.	Percentage transmission without F.A.D.	Percentage transmission with F.A.D.	
				$FeCl_3$ last technique	Dipyridyl last technique
3	Groundnut oil :				
	(*a*) Before heating	25	56	60	89
		25	41	46	76
		50	56	66	91
		50	41	52	85
	(*b*) After 6 hrs. heating	25	56	65	93
		25	41	50	88
		50	56	68	96
		50	41	56	93
	(*c*) After 9 hrs. heating	25	56	66	92
		25	41	51	89
		50	56	68	95
		50	41	55	94
	(*d*) After 19 hrs. heating	25	54	72	96
		25	42	59	93
		50	54	78	96
		50	42	69	93
	(*e*) After 25 hrs. heating (rancid oil)	25	53	93	96
		25	38	88	93
		50	53	92	96
		50	38	90	95

60 minutes after making up to volume (10 cc.). The results, given in Table IX, show that free fatty acids, saturated and unsaturated, give a positive reading depending on the nature and amount of fatty acid present. Hence presence of these can cause positive error in the direct determination of tocopherol and extracted oils must be freed from fatty acids before estimation. Purified ethyl stearate did not produce any colour but purified ethyl oleate did ; the reason for this is not quite clear. However, purified fully saturated glycerides and groundnut oil did not give any colour at all and hence the neutral glycerides will not produce any error in the direct estimation of tocopherol.

It also became evident during these studies that inhibition can be produced by various other materials ; mineral acids like hydrochloric and sulfuric can produce inhibition when present in appreciable quantities, i.e. a mg. or more ; but these are not likely to be present during the above estimations. Alkali metal salts of organic acids also produced inhibition, e.g. potassium acetate produced inhibition at very small concentrations and other organic acids giving water soluble alkali metal salts may also behave similarly. Since solvents can extract traces of such soaps from crushed seeds when present, it is essential to wash the crude extract with water to free it from water soluble constituents.

TABLE IX

Development of colour on treating purified F.A.D. with Emmerie and Engel reagent

(Cell 1 cm. : Filter M 515)

Serial No.	Nature of F.A.D.	Wt. of F.A.D. mg.	Percentage transmission against reagents alone	
			after 15 min.	after 60 min.
1	Saturated acids from *Myristica malabarica*	100	85	83
		50	89	89
		25	94	94
		10	96	97
		5	98	99

TABLE IX—(contd.)

Development of colour on treating purified F. A. D. with Emmerie and Engel reagent

(Cell 1 cm. : Filter M 515)

Serial No.	Nature of F. A. D.	Wt. of F.A.D. mg.	Percentage transmission against reagents alone	
			after 15 min.	after 60 min.
2	Stearic acid (pure)	100	88	86
		50	93	91
		25	95	94
		10	97	96
3	Unsaturated acids from groundnut oil	100	84	75
		50	87	79
		25	91	86
		10	99	96
4	Ethyl oleate	100	94	83
		50	97	91
		25	99	96
		10	100	99
5	Ethyl stearate	100	100	100
		50	100	100
6	F.S.G. from coconut oil	100	100	99
		50	100	99
7	Groundnut oil (purified)	100	99	98
		50	100	99

It has been mentioned earlier that during alkali saponification of oils part of the tocopherol gets oxidised ; it has hence to be verified whether alkali washing in ethereal solution will produce any such destruction. For this a known amount of a deacidified oil of known tocopherol content was dissolved in pure ether, shaken with excess of 1 per cent KOH solution for 15 minutes, washed free of alkali and the tocopherol content of recovered oil determined ; this was exactly the same as that of the material started with. Hence no appreciable loss of tocopherol can take place during treatment of extracted oils with alkali to remove acidic impurities.

On the basis of the above results the following procedure was drawn up to estimate the tocopherol content of oil in fresh seeds under conditions which produce minimum peroxidation of fats :

0·2—0·3 gm. of fresh seeds are mixed with 2 gm. anhydrous sodium sulfate and 2-3 gm. of powdered glass and ground well in a mortar. This mixture is then transferred to a small glass percolator and packed over a $\frac{1}{4}$ inch thick bed of anhydrous sodium sulfate supported on a wad of cotton wool and percolated with 30—35 cc. of carefully purified sulfuric ether. The extraction of oil would be practically complete by this time. The percolate is transferred to a separating funnel and washed first with water and then with dilute aqueous potassium hydroxide and subsequently washed till neutral. The ethereal solution is dried over anhydrous sodium sulfate and solvent removed and weight of residual oil determined. The oil is dissolved in a mixture of carefully purified benzene and alcohol mixture (2:1) (*loc. cit.*) so that 1 cc. contains 20 mg. and then 2 cc. of this solution is taken for each determination; the estimations are finished within an hour of the extraction.

When the oil is extracted from the seed as mentioned above it is found that there is practically no inhibition since when a known amount of tocopherol is added to the solution the increase in E.C. corresponds exactly to that produced by the same amount of tocopherol in absence of any fat. However, if the extracted oil is allowed to remain in contact with air for some time or in case the seeds are damaged and perhaps if they are too old, inhibition appears. Hence in each case extracted fat has to be tested for inhibition by adding a known amount of tocopherol and comparing the increase in E. C. to that theoretically required. It was also found that in case of freshly extracted and purified fat not showing inhibition by the ferric chloride last technique, no inhibition was present even when the colour was developed by the dipyridyl last technique as previously described, and this eliminates possibility of any error by the local concentration of ferrous chloride during reaction, and other factors. Incidentally this forms a simple method for qualitatively testing whether any inhibition is being exerted by any extraneous substances present along with the tocopherol ; presence of inhibition will result in different E.C.s when estimations are done by the ' ferric chloride last ' and ' dipyridyl last ' techniques ; in the absence of any inhibitive effect the same E.C. will be developed by both techniques.

The free tocopherol contents of a series of vegetable oils were determined by the above procedure and are given in Table X. These represent the most accurate values obtained for these so far, for in all these cases the oils were examined when quite fresh.

In view of the fact that an accurate estimation of free tocopherol in fresh oils could be made, an attempt was made to estimate the total tocopherol also by the same procedure. It was investigated whether fatty acids prepared by hydrolysis from freshly extracted oils produced any inhibition; they did not produce any inhibition when fresh and added amounts of tocopherols could be quantitatively estimated. However, when fresh oils were hydrolysed by alcoholic alkali and the amount of tocopherol in the fatty acids estimated after making a due correction for the colour produced by fatty acids, it was found that some destruction of the free tocopherols takes place ; the total tocopherol values after hydrolysis being frequently lower than free tocopherol values before hydrolysis. Even when hydrolysis is

effected in an atmosphere of hydrogen by adding sodium to a solution of oil in alcohol-benzene mixture and the reaction products subsequently worked up, the above loss of tocopherol does not always disappear. It is hence to be concluded that destruction of tocopherol during alkali saponification probably does not take place by oxidation by atmospheric oxygen alone but may also be induced by oxidising agents present in the fats themselves. Perhaps it may be that small quantities of peroxide may be present even in freshly extracted oils : and though these may not be able to produce any measurable inhibition, may liberate nascent oxygen in contact with alkali during saponification and this may cause the destruction of tocopherols. Exclusion of atmospheric air or working in carbon dioxide or nitrogen atmospheres cannot help much in preventing tocopherol destruction due to this source and methods not involving alkali (or acid) saponification may have to be developed before any accurate idea of the small amounts of esterified tocopherol present in oils and fats can be obtained.

TABLE X

Tocopherol contents of some fresh vegetable oils

Serial No.	Oil	Percentage transmission for 40 mg. oil (Cell 1cm. : Filter M 515)	E.C. for 40 mg. oil	Percentage Tocopherol content
1	Groundnut oil	85	0·0706	0·050
2	Sesame oil, mixed (1955) specimen	77	0·1135	0·078
3	Sesame oil, white (1955) specimen	82	0·0862	0·058
4	Sesame oil (1952) specimen	78	0·1079	0·075
5	Sesame oil, N.P. 29 (1952)	82	0·0862	0·058
6	Poppyseed oil (1952) specimen	95	0·0223	0·015
7	Mustard seed (1952) Rai variety	62	0·2076	0·140
8	Mustard seed (1952) specimen	76	0·1192	0·080
9	Tobacco seed (1952) specimen	77	0·1135	0·078
10	Melon seed	77	0·1135	0·078
11	*Kheera* seed	76	0·1192	0·080
12	Safflower seed (1952) specimen	89	0·0506	0·035
13	Water melon-seed	80	0·0969	0·065
14	*Chilgosa* seed	78	0·1079	0·075
15	Walnut seed	78	0·1079	0·075
16	Linseed N.P. 12 (1952) specimen	78	0·1079	0·075
17	Linseed N. P. 236 (1952) specimen	76	0·1192	0·080
18	Sapota seed	92	0·0362	0·024
19	Cashewnut oil	90	0·0458	0·031
20	Coconut oil	98	0·0088	0·006

Summary

Methods of estimation of tocopherol involving saponification of fats and extraction of unsaponifiables are recognised to be somewhat inaccurate due to various sources of error which cannot be eliminated. The direct estimation of free tocopherols in oils has hence been investigated in detail.

The nature and mechanism of inhibition in the direct estimation of tocopherol in oils and fats by the Emmerie and Engel's method was studied. Inhibition is produced only by unsaturated acids and their derivatives and it is caused by the oxidising action of fat peroxides usually present in these ; the degree of inhibition produced by any oil will hence vary with its peroxide content. Alkali metal salts of some organic acids also inhibited the colour formation to some extent. Free fatty acids, saturated as well as unsaturated, even when carefully purified from last traces of tocopherol produced a certain amount of colour on mixing with the Emmerie and Engel reagent ; this colour is, however, additive. Degree of inhibition produced by fats is lower when fats are comparatively fresh, and when undamaged seeds are extracted in the cold with sulphuric ether freed from peroxides, the resulting fats show no inhibition at all. On the basis of the above results a rapid method has been developed for direct estimation of free tocopherols using *ca.* 0·1 to 0·3 gm. of seeds or about 40 mg. of oil. The free tocopherol contents of some 20 different varieties of common Indian oils determined by the above method are given.

A simple method has been developed for the qualitative detection of inhibition.

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DETERMINATION OF SATURATED ACIDS BY DIRECT ACETIC ACID-ACETONE-PERMANGANATE OXIDATION OF MIXED FATTY ACIDS

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In the study of the metabolism of fats a rapid and accurate method for direct determination of saturated acids in small quantities of mixed fatty acids will be of great utility. Such a method has, however, not so far been evolved. Estimations based on separation of saturated acids as different metal soaps, are at best only approximate. Conversion of the acids to esters followed by acetone-permanganate oxidation by the method of Armstrong and Hilditch [1925] produces a number of stages. This lengthens considerably the time required and may lower the accuracy by loss at various stages. Direct alkaline permanganate oxidation below 25°C as reported by Bertram [1925, 1927, 1928] during which large proportions of unsaturated acids remain at the hydroxylated stage, does not give accurate results always since in the separation of the saturated acids from the latter by petroleum ether, intersolubility effects can produce errors as shown by the results of Hilditch and Priestman [1931]. Raising the temperature of oxidation to 50°C produces a decrease in the amount of hydroxy acids [Hilditch and Priestman, 1931]. Further, even at 60°C about 60 per cent of oleic acid remains at the dihydroxy acid stage [Edmed, 1898; Lapworth and Mottram, 1925] and hence this does not increase the the accuracy very much. Increasing the temperature of oxidation beyond 50°C is feared to lead to destruction of saturated acids as well [Hilditch and Priestman, 1931], but no clear cut idea of the extent of destruction involved seems to have been recorded in the literature.

To study this 500 mg. of stearic acid of iodine value 0·0 was dissolved in 150 ml. of 3 per cent aqueous NaOH and oxidized by the addition of 250 ml. of a 3 per cent solution of $KMnO_4$. The mixture was kept on a water-bath at 95-96°C for 15 hours with frequent shaking. In between, the acid remained in contact with $KMnO_4$ for nearly 50 hours at about 30°C. At the end of the period only 60 mg. of stearic acid was recovered by the method outlined by Kartha [1954]. This shows that heavy degradation of the acid occurs during alkaline permanganate oxidation at elevated temperatures.

Przheval'skii [1911] reported that direct acetone-permanganate oxidation of caproic, enanthic and other saturated monocarboxylic acids gave rise to breakdown products. Levene and West [1914] have, however, reported that 2-hydroxy-stearic acid and palmitic acid are oxidized mainly to margaric and pentadecanoic acids by this procedure with relatively little further degradation. This difference may

be due to greater susceptibility of the lower acids. During studies on the oxidation of fatty acids with hydrogen peroxide Dakin [1907] and Allen and Witzeman [1941] showed that saturated monocarboxylic acids were apparently quite resistant to the action of hydrogen peroxide in the absence of catalysts. Dakin [1907], Witzeman [1918, 1920, 1921, 1927] and Spoehr [1924] showed that in presence of alkaline catalysts, e.g. ammonia, glycine, etc. heavy oxidation took place, acetic acid and acetone being the intermediate products of oxidation. This indicates that it is the salts of the acids which are degraded rather than the acids themselves. Whether this interesting behaviour of saturated acids towards oxidising agents applies to other techniques of oxidation also has not been systematically investigated so far. In the ordinary acetone-permanganate oxidation [Przheval'skii, 1911; Levene and West, 1914] alkaline conditions are present due to alkali developed during oxidation and the behaviour of saturated monocarboxylic acids to acetone-permanganate oxidation in presence of excess of acetic acid, which prevents the possibility of any of the higher acids being converted to soaps, has now been studied. The results showed that whilst lower saturated acids may be reactive to acetone-permanganate oxidation, the higher ones are not affected when the reaction is carried out in acetone-acetic acid using the limited amount of $KMnO_4$ (maximum 12-15 gm. per gm. of fatty acid) that is necessary to break down the unsaturated bonds. The possibility of using this principle to estimate higher saturated acids has been investigated systematically in the course of the present investigation.

Experimental

A definite indication that the higher saturated acids were not broken down in any measurable quantity during acetic acid-acetone-permanganate oxidation was obtained when 500 mg. of pure stearic acid (*loc. cit.*) were oxidized by the procedure to be described subsequently. 7 gm. of $KMnO_4$ was used up during the oxidation but 497-499 mg. of stearic acid (duplicates) were recovered after the oxidation. Hence this technique can satisfactorily be used for breakdown of unsaturated members in mixed acids provided that, as in the case of acetic acid-acetone-permanganate oxidation of fats, no intermediate hydroxylated products of oxidation of unsaturated acids are present in the oxidised material. This has been verified in all the experiments described subsequently by dissolving the saturated acids finally obtained in small volumes of petroleum ether b.p. 40-60°C ; in all cases clear solutions were obtained showing complete absence of hydroxylated products.

It has been shown in an earlier communication by Kartha and Sethi [1956] that when the fat is oxidised by this technique, and the products of oxidation submitted to Bertram separation under modified conditions using the same volume of reactant solutions as required for 5 gm. fat [Kartha, 1954], the yield of saturated acids is unaffected by variation of the weight of the sample from 1 gm. to 6 mg.

Hence the final technique evolved for the oxidation of mixed fatty acids is as follows : *ca.* 1 gm. of mixed fatty acids is dissolved in 100 ml. of acetone. 12 ml. of acetic acid are added (150 ml. acetone and 15 ml. acetic acid are used when iodine value of fatty acids is above 100) and oxidised with powdered permanganate, the latter being added in small lots of *ca.* 2 gm. at a time, while the reaction mixture is

gently refluxed under a reflux condenser on a water-bath. In the case of fatty acids with iodine value less than 100 units 10 gm. of permanganate are used ; where iodine value is above 100, 12 gm. of permanganate are used. The refluxing is continued for a total period of 8 hours and then the reaction mixture worked up irrespective of whether all the permanganate has been used up or not. The products of oxidation are extracted with ether, solvent removed and dissolved in hot aqueous ammonia and directly submitted to magnesium salt separation twice as already described [Kartha, 1954], same volumes of reactant solutions are used as for 5 gm. samples as in the earlier report. The fatty acids are recovered, weighed, and a correction made to allow for their observed iodine values. The per cent saturated acids in mixed fatty acids in six fats which had been analysed by direct oxidation of fat are given in Table I wherein the results obtained on the fat basis in the latter method have been corrected to allow for the glycerol content. It is seen that the results by the present technique agrees very closely with the results by the fat oxidation method.

TABLE I

Saturated acids per cent in some fats by different methods

Fat	Per cent saturated acids in mixed acids	
	Mixed acid oxidation method (Present method)	Fat oxidation method
Garcina indica fat	65·9	66·1
Vateria indica fat	60·1	59·2
Beef tallow (New zealand)	56·7	57·2
Lard (Denmark)	46·9	47·6
Groundnut oil	18·7	19·6
Sesame oil	17·4	16·6

Apart from its utility in studies on metabolism of fats, the present method is also of use in the accurate estimation of linoleic acid in small quantities of oils. This is of importance from the point of view of the plant breeder, for in the case of edible oils and fats, the stability is to a large extent determined by the linoleic acid content [Hilditch and Gunstone, 1946] and the breeding of varieties of edible seeds with lower linoleic acid content is a distinct advantage. Several methods have been reported in the literature for determination of linoleic acid content, the most recent being the spectrophotometric technique but many or most of these suffer from the drawback that they are based on empirical values worked out for pure linoleic acid isolated from fats. The linoleic acid thus isolated would represent only the most predominant octadecadienoic acid present and any isomer present in small quantities will be entirely left behind during the process of isolation of pure acid and it is by no means improbable that the isomers may behave differently from the predominant constituent in this respect. The presence of small amounts of solid isomers of oleic

acid in nearly all natural oils and fats has recently been demonstrated by Narayanan and Kartha [1956] and this makes it quite probable that linoleic acid may also occur in more than one form. An example that spectrophotometric method is not fully reliable in quantitative determinations is evident from the results of Luddy *et al.* [1954]. These workers determined the saturated acid contents of different specimens of fats by the oxidation method of Kartha [1951, 1953] (I) as well as by the difference method where the different unsaturated acids are estimated by the spectrophotometric technique (II), for chicken fat, cottonseed oil and palm oil the saturated acid contents were 29·2, 24·6 and 51·2 by (I), while the values were 30·4, 26·7 and 54·6 by (II). Kartha and Sethi [1956] have shown that during the oxidative determination of saturated acids no loss of the latter occurs due to solubility of magnesium soaps of higher acids and hence the higher values for saturated acids by the spectrophotometric technique must be due to errors in the technique followed. The determination of iodine values and saturated acid contents of mixed fatty acids freed from unsaponifiables thus forms the most reliable method for determination of linoleic acid content of edible oils and for this, the present technique can be used with advantage. The per cent linoleic acid content in mixed acids from a number of oils and fats have been determined by this method and are given in Table II ; for purposes of calculations it has been assumed that fats numbers 1-12 do not contain any acid more unsaturated than linoleic acid.

TABLE II

Linoleic acid content in mixed acids from some oils and fats

Fat from which mixed acids are derived	Per cent saturated acids (Present method)	Iodine value mixed acids	Per cent Oleic acid	Per cent Linoleic acid
Garcina indica fat	65·9	33·0	31·6	2·5
Vateria indica fat	60·1	37·4	38·3	1·6
Beef tallow (New zealand)	56·7	40·0	42·2	1·1
Lard (Denmark)	46·9	54·5	45·8	7·3
Mowhra oil	43·2	62·0	44·9	11·9
Hen fat (Indian)	36·1	73·7	46·2	17·7
Erithrina indica fat	34·9	72·2	50·2	14·9
Memusops elengi fat	31·6	85·2	42·6	25·8
Water-melon	21·6	131·6	11·6	66·8
Groundnut oil	18·7	97·2	55·0	26·3
Sesame oil	17·4	122·8	29·6	53·0
Olive oil	16·3	87·8	70·0	13·7
Linseed oil	10·8	174·3	..	..

Summary

It has been shown that heavy degradation of stearic acid occurs during aqueous alkaline permanganate oxidation at 95-96°C, saturated fatty acids undergo similar degradation on oxidation with permanganate in acetone solution. A new technique has been developed which eliminates such changes. The technique has been successfully used for the oxidative destruction of unsaturated acids in mixed acids,without affecting higher saturated acids.

A new procedure is outlined to determine the saturated acid content in small amounts of mixed fatty acids by acetic acid-acetone-permanganate oxidation and Bertram separation of the oxidation products. Comparative results for six fats are given. The saturated acid content of a number of natural fats has been determined by this procedure.

The technique also provides a rapid method for accurate determination of linoleic acid in small quantities of mixed fatty acids.

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EFFECT OF DIFFERENT TIMES OF APPLICATION OF AMMONIUM NITRATE ON AMAN PADDY ON GANGETIC ALLUVIAL SOIL

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NITROGEN being a basic part of proteins and chlorophyll is essential for cell formation in plant growth. It encourages the development of foliage and tends plumpness in the seeds. The soils of India are very deficient in this element, and the manurial problem in India is mainly one of nitrogen deficiency.

This deficiency can be partially made up by application of natural and artificial nitrogenous manures and fertilizers. The available natural manures in this country are insufficient in amount and sometimes uneconomical also. The use of artificial fertilizers has, therefore, to be resorted to. Of the different nitrogenous fertilizers, ammonium sulphate is preferred because of its established use and superior physical properties. Its popularity has also been enhanced by its results in increasing the yield of crop, in large number of manurial experiments. A factory for the manufacture of this fertilizer has been established at Sindri and establishment of more factories for the production of synthetic nitrogenous fertilizers has been envisaged in the Second Five-Year Plan.

Ammonium sulphate is being manufactured at present by double decomposition with gypsum. The deposits of gypsum occuring in Rajputana are being utilised for this purpose. Cement and paint industries are other major consumers of gypsum. Demands for gypsum from these industries may keep the price of gypsum sufficiently high to defeat all attempts to bring the price of ammonium sulphate down to suit the economy of the falling food prices. The stock of gypsum in the country is also not very promising. We have no other source of suitable sulphur bearing compounds also. These stand against the prospect of ammonium sulphate being the only nitrogenous fertilizer in India.

The suggestions for alternative fertilizers favour the production of ammonium nitrate, urea and ammonium chloride. Among these, ammonium nitrate is the most important. Its synthetic production is not difficult. It can become cheaper product than ammonium sulphate in consideration of the fact that the sources of raw materials for the latter are distributed over widely separate places. Ammonium nitrate has also been used abroad and its performance under those conditions is known. Urea and ammonium chloride have not, however, been largely used as a fertilizers either abroad or in this country yet.

Ammonium nitrate has certain properties, which militate against its use. It is hygrospic and cakes in damp weather. It may decompose, sometimes explosively, when subject to contamination with easily oxidizable substances. Methods have,

however, been developed for producing the fertilizer in granular form. The granules then are protected by oiling and dusting with kieselguhr. If stored in such condition in moisture proof containers, this fertilizer will keep reasonably well in our climatic conditions. The production of nitrochalk may be the other method to considerably overcome its hygroscopicity and it is well adopted to our soils, especially which are low in lime.

However, from the standpoint of plant food, it has given an excellent account of itself, because it contains nitrogen both in the ammonia and nitrate forms. Over and above, the equivalent acidity as judged in terms of neutralisation by calcium carbonate is 60 with this fertilizer, whereas 110 is the value for ammonium sulphate. This is, therefore, less acid forming and, therefore, safer than ammonium sulphate so far as development of soil acidity is concerned.

Review of past work

Skinner and Buie [1926] studying the comparative performances of ammonium sulphate and ammonium nitrate on the yield of cotton and corn observed that on a sandy loam soil of South Carolina, both these fertilizers produced equally good results. Skinner, Williams and Mann [1935] found that the yield of cotton and sweet potato could be increased by the application of ammonium nitrate. Borden [1929] comparing the effect of ammonium sulphate and ammonium nitrate on the yield of Sudan grass found that the effectiveness of ammonium nitrate decreased with the increased *p*H value of the soils. On soils with *p*H 5·6, ammonium nitrate was better than ammonium sulphate; at *p*H 5·6 both the fertilizers were identical; at *p*H 7·1, ammonium sulphate became better than ammonium nitrate. Paden [1939] also observed that on soils with *p*H value from 6·0 to 6·5, ammonium sulphate and ammonium nitrate were identical in their effects on the yield of cotton. Skinner [1944] found that in the sandy loam soil in North and South Carolina, ammonium nitrate produced yields equal to ammonium sulphate. Sen and Sher Singh Bains [1952] on soil under Delhi and Karnal conditions with *p*H values 7·3 and 8·1 respectively, found that ammonium nitrate alone or admixtured with trinitrotoluene (T.N.T.) is as good a fertiliser as ammonium sulphate for crops like sorghum, wheat, maize, cotton and paddy. In the Rice Research Station at Chinsura (*p*H6·8-7·0), West Bengal (1945-48), ammonium sulphate has been found to be slightly better than ammonium nitrate in increasing yield of paddy, both grain and straw. Anderson, Jones and Armiger [1946] compared the effect of urea, and ammonium nitrate with ammonium sulphate on *aman* paddy. They observed that ammonium portion of the nitrogen present in ammonium nitrate was as good as the best nitrogen carriers. Ammonium sulphate acted as the most efficient nitrogenous fertilizer and urea was often preferable to ammonium nitrate. Ramiah, Ghose and Vachhani [1952] summarised the fertilizing effect of different nitrogenous fertilizers on the yield of paddy. They observed that application of 20-30 lb. N per acre increased grain

yields by 300-500 lb. and compost at 4 tons/acre had additional effects. The relative efficiency of the different nitrogenous fertilizers was:

Ammonium sulphate—100
Ammonium phosphate—86
Calcium cyanamide—64
Sodium nitrate—40
Ammonium nitrate—92
Urea—82
Potassium nitrate—44

Thus, it may be seen that in view of its limited use much work has not been done on various crops, especially on paddy. Considering that ammonium nitrate may in future have an important role as a nitrogenous fertilizer in this country, attempt has been made to find out a suitable dose of this fertilizer and the best time of its application for *aman* paddy, which is the most important crop of West Bengal.

The site for this experiment had been selected in the State Agricultural Farm at Chinsura, West Bengal. The soil of the experimental site was a typically paddy growing one and was unconsolidated gangetic alluvium. The clay content of the top soil is about 55 per cent, which gradually decreases with depth downwards. Leaching of calcium has taken place and the soil below the depth of 1 ft. contains free calcium carbonate. Description of typical profile, from the experimental plot is given in Table I.

TABLE I

Results of analysis of a soil profile taken at 1 ft. depth in the experimental area
(*On oven-dry basis*)

Constituents (per cent)	Depth in inches				
	0-12	12-24	24-36	36-48	48-60
R_2O_5	21·44	19·79	15·97	18·78	11·23
Al_2O_5	36·68	12·51	9·33	11·26	11·5

TABLE I (contd.)

Results of analysis of a soil profile taken at 1 ft. depth in the experimental area (On oven-dry basis)

Constituents (per cent)	Depth in inches				
	0-12	12-24	24-36	36-48	48-60
CaO	8·84	1·48	1·22	2·42	2·46
P_2O_5	0·089	0·105	0·099	0·117	0·168
K_2O	0·86	0·54	0·44	0·36	0·46
N/2 Acetic acid (m.e. soluble calcium.)	19·00	30·80	37·97	51·40	66·40
N/2 Acetic acid soluble bases (m.e.).	22·70	38·00	50·80	62·40	70·20
Coarse sand	0·29	·52	0·45	0·39	0·21
Fine sand	6·53	11·60	4·99	17·78	10·46
Silt	30·00	33·45	39·55	35·45	42·75
Clay	55·75	53·15	48·15	43·30	43·70
Moisture	7·35	3·50	5·04	4·40	4·36
pH	6·80	7·40	7·60	7·60	7·80
Nitrogen	0·080	0·066	0·041	0·042	0·041
Carbon	0·75	0·48	0·35	0·26	0·36

The normal annual rainfall in the locality is about 58 inches. During the years 1948, 1949, 1950 and 1951, the total rainfall had been 58·18, 77·79, 57·15 and 41·93 inches respectively.

On the experimental site, transplanted *aman* paddy was grown year after year and for five years before the experiment under review; practically no manure was applied.

Experiment

The experiment was designed to have 12 treatments entailing all combinations of 4 doses of nitrogen as ammonium nitrate, e.g., 0,20,40 and 60 lb. N per acre and 3 times of application, e.g. (*a*) full dose at puddling, (*b*) half dose at puddling, half dose 4 weeks after transplantation and (*c*) full dose 4 weeks after transplantation. These 12 treatments were replicated 4 times in randomised blocks.

The experiment was conducted for 4 years in succession in the same field from 1948 to 1951. The variety of paddy used in this experiment was transplanted *aman* paddy, variety being *Bhasamanik*.

The land was prepared in usual manner with the break of monsoon. The soil was puddled and ammonium nitrate applied to different sub-plots as detailed in the plan of layout. Seedlings were transplanted at the rate of 2 per hole. Care was always taken to keep the plant population same in each sub-plots. Ammonium nitrate was then applied during different periods as per timings of application under test. No basal dose of manure or fertiliser was applied.

The effect of the different treatments on the growth of the plants were studied for (*a*) height of the plants, (*b*) number of tillers per plant and (*c*) incidence of pests and diseases. It was found that both height of the plants and the number of the tillers per plant increased with the application of ammonium nitrate. Incidence of pest and diseases, if took place were erratic and had no correlation with the treatments.

During harvesting, a border of one feet was left out and the plants within the net area of a plot were harvested. After harvesting, the crop was allowed to dry for about a month and then threshed. The grain yield and straw yield are given on air-dry-basis.

Results

The yields of grain per plot for each year is given in Table II and of straw in Table III. The treatment effects for the different years have been separated, statistically examined, and are given in Tables IV(*a*) and IV(*b*). The results have also been consolidated and the mean yields of grain due to different treatments and the corresponding critical differences at different levels have been given in Table V.

TABLE II

Grain yield per net plot (in seers)

(Gross area of each sub-plot—34 ft. × 19 ft. Net area of each sub-plot—32 ft. × 17 ft.)

BLOCK-I	BLOCK-2				BLOCK-3				BLOCK-4		
A4	C2	A1	C2	B1	I I	A1	A2	B2	C1	C2	A1
1. 7·00	6·0	8·25	9·00	7·25	12·00	9·75	· 25	10·50	7·25	6·58	7·25
2. 15·25	14·00	10·75	14·50	11·25	15·00	11·50	15·00	13·75	11·25	14·00	10·25
3. 13·62	13·62	10·75	13·31	8·75	11·75	8·75	8·36	11·31	10·00	10·00	9·75
4. 5·25	2·5	2·69	3·56	3·25	5·06	3·25	5·31	4·12	2·06	2·50	3·06
A2	C1	B4	A3	B2	C4	C4	B1	A4	A3	B1	A4
1. 4·25	5·75	9·75	7·75	9·50	11·75	10·25	7·75	11·25	10·75	5·62	11·25
2. 13·00	13·00	1 5·00	16·25	14·25	15·00	16·50	11·75	15·00	14·00	9·50	15·00
3. 13·50	9·25	16·50	13·12	9·00	12·50	14·31	8·75	14·12	13·00	10·00	12·00
4. 3·50	2·50	6·31	5·94	3·69	4·00	5·06	3·50	6·50	0·6	2·06	6·25
B1	B3	C3	C1	A4	A1	C1	B4	C2	A2	B2	B3
1. 5·50	6·25	8·50	6·25	14·00	8·50	4·00	10·25	8·50	9·62	8·25	9·75
2. 12·50	13·50	13·75	13·75	15·50	9·75	11·25	15·25	13·75	11·75	11·25	15·75
3. 8·25	15·00	12·87	10·00	13·25	7·36	10·50	13·00	9·87	12·75	11·00	12·00
4. 2·31	6·25	3·06	3·06	6·50	3·56	3·94	4·62	4·62	4·25	2·50	3·12
B2	C4	A3	A8	C3	B4	B3	A3	C3	C3	C4	B4
1. 6·50	5·50	7·75	11·25	12·25	11·00	9·50	12·25	9·75	10·50	12·75	12·25
2. 11·75	13·50	13·25	14·75	15·25	15·00	15·25	15·00	13·75	12·25	14·00	13·00
3. 12·36	13·36	14·12	12·00	12·25	14·36	14·25	12·25	12·69	12·75	12·12	14·36
4. 5·25	4·50	5·00	4·62	5·19	4·69	6·19	7·19	4·81	3·44	4·25	2·81

Symbols indicating the treatments per acre :

	0 lb. N	20 lb. N	40 lb. N	60 lb. N
Full dose of ammonium nitrate at puddling.	A1	A2	A3	A4
Full dose of ammonium nitrate, 4 weeks after transplantation.	B1	B2	B3	B4
Half dose of ammonium nitrate at puddling and half dose, 4 weeks after transplantation.	C1	C2	C3	C4

Year

1. 1948
2. 1949
3. 1950
4. 1951

Table III

Straw yield per net plot (in seers)

(Gross area of each sub-plot—34 ft. × 19 ft. Net area of each sub-plot—32 ft. × 17 ft.)

BLOCK-1	BLOCK-2				BLOCK-3				BLOCK-4		
A4	C2	A1	C2	B1	B3	A1	A2	B2	C1	C2	A1
1. 8·62	8·75	11·62	11·25	9·69	14·56	10·69	12·62	13·62	11·62	10· 2	9·6
2. 23·50	21·50	16·00	20·00	16·00	23·50	19·00	22·50	20·50	16·00	20·50	14·00
3. 18·50	16·12	13·25	14·50	10·50	13·00	12·36	12·12	15·00	12·50	12·62	12·62
4. 9·75	7·36	5·50	8·56	4·31	9·00	9·25	10·36	8·50	3·25	8·00	5·00
A2	C1	B4	A3	B2	C4	C4	B1	A4	A3	B1	A4
1. 7·75	7·75	14·50	13·56	12·62	15·50	14·50	8·75	14·50	16·50	9·75	17·00
2. 20·00	18·50	26·50	28·00	22·50	24·50	26·50	19·00	25·50	22·50	13·50	25·00
3. 16·25	23·50	19·50	16·50	12·35	15·00	20·87	10·75	17·50	16·00	12·00	16·62
4. 6·75	5·62	9·56	10·75	7·25	11·00	12·00	7·44	12·00	7·50	6·00	9·25
B1	B3	C3	C1	A4	A1	C1	B4	C2	A2	B2	B3
1. 8·75	10·12	12·62	7·50	17·00	10·12	11·56	12·87	0·75	13·50	14·50	15·12
2. 17·00	22·50	24·00	21·50	26·00	15·00	19·00	23·50	19·50	19·50	18·00	20·00
3. 10·75	18·50	15·87	13·00	17·00	10·62	13·56	16·25	12·75	15·25	14·75	15·75
4. 6·69	10·00	9·00	16·00	12·31	5·69	7·06	10·00	8·25	5·25	7·44	11·25
B2	C4	A3	A2	C3	B4	B3	A3	C2	C3	C4	B4
1. 12·62	12·62	11·62	12·12	15·50	14·50	13·12	13·62	12·62	15·50	18·37	17·12
2. 19·00	21·50	21·50	24·00	24·00	23·50	26·50	25·50	22·50	20·50	23·00	24·00
3. 14·50	16·50	17·00	15·36	15·12	17·75	19·62	15·12	15·50	15·00	16·50	17·50
4. 7·00	10·75	9·25	10·12	10·00	7·25	10·25	10·36	10·50	8·56	12·25	8·75

TABLE IVA

Effects of 'time of application' on grain and straw yields (in md. per acre) (single factor effects)

Year	Full dose at puddling	Half dose at puddling, half 4 weeks after	Full dose 4 weeks after trans-plantation	'F' test (time of application)	Standard error (Av. time)	Critical difference (Av. time)	
						at 5 per cent level	at 1 per cent level
Grain	19·73	19·25	18·54	Not signi-ficant			
1948—							
Straw	26·40	27·54	26·43	,,			
Grain	28·96	28·12	28·21	,,			
1949—							
Straw	47·25	45·00	44·67	,,			
Grain	25·35	25·82	24·94	,,			
1950—							
Straw	32·20	32·41	31·06	,,			
Grain	10·73	9·10	7·92	**	±0·48	1·40	1·88
1951—							
Straw	18·94	17·71	19·37	Not signi-ficant			

**Significant at 1 per cent level.

TABLE IVB

Effects of 'nitrogen' on grain and straw yields (in mds. per acre)

Year	0 lb. N per acre	20 lb. N per acre	40 lb. N per acre	60 lb. N per acre	'F' test (Nitrogen)	Standard error (Av. nitro-gen)	Critical difference (Av. nitrogen)	
							5 per cent	1 per cent
Grain	13·85	16·85	19·50	21·16	**	±0·96	2·74	3·68
1948—								
Straw	19·58	23·45	27·41	29·52	**	±0·88	2·54	3·41
Grain	22·75	26·79	28·83	29·67	**	±0·60	1·70	2·29
1949—								
Straw	34·08	41·25	46·83	48·83	**	±1·01	2·90	3·90
Grain	18·68	22·85	16·01	27·25	**	±0·71	2·03	2·73
1950—								
Straw	24·04	28·59	32·16	34·92	**	±0·85	2·44	3·23
Grain	5·87	7·74	9·56	10·12	**	±0·44	1·26	1·69
1951—								
Straw	11·97	15·81	19·40	20·81	**	±0·66	1·91	2·57

**Significant at 1 per cent level.

TABLE V

Consolidated main yield of grain and critical differences
(Yield in md. per acre)

Dose of nitrogen (N)	0 lb. N per acre	20 lb. N per acre	40 lb. N per acre	60 lb. N per acre	Av. T. (S.E. ±0·33)
Time of application (T)	..	..	..	..	..
A. Full dose at puddling	..	19·40	21·46	22·72	21·19
B. Half dose at puddling	..	18·12	21·33	22·27	20·57
C. Full dose 4 weeks after transplantation	..	18·15	20·38	21·17	19·90
Av.N (S.E. ±0·32)	15·29	18·56	21·06	22·05	..

S. E. in the body of the Table—0·58.

F test for 'N'—Significant at 1 per cent level.

F test for 'time of application'—Significant at 5 per cent level.

Critical difference for dose of 'N' at 5 per cent level-0·92 ; at 1 per cent level-1·24.

Critical difference for 'time of application' at 5 per cent level-0·97 ; at 1 per cent level-1·30.

DISCUSSION OF THE RESULTS

(a) *Effect of treatments on the yield*

Grain yields increased significantly at 1 per cent level with the increasing doses of nitrogen up to 40 lb. N per acre. The increases in yield produced by 60 lb. N over no nitrogen and 20 lb. N per acre are significant at 1 per cent level, but that over 40 lb. N is just significant only at 5 per cent level and thus is of comparatively less significance.

Straw yields significantly increased with the increasing doses of nitrogen up to 40 lb. N per acre, beyond which no further significant increase could be observed.

(b) *Effect of time of application on the yield*

The yield of grain obtained by the application of full dose of ammonium nitrate at puddling was significantly higher at 5 per cent level than that produced by applying the full dose of the fertilizer 4 weeks after transplantation. It may be noted that the above mentioned difference occurred only in the year 1951-52, the year of very low rainfall, resulting in similar difference in the consolidated mean yield. In other years, however, no difference had been observed among the different times of application even at 5 per cent level. In none of the years, the different times of application had produced significant differences in the yield of straw both at 5 per cent and 1 per cent level.

(c) *Straw to grain ratio*

The straw to grain ratio was calculated and the results are presented in Table VI below.

TABLE VI

Straw and grain ratio under different treatments

Year	Rainfall in inches	Straw to grain ratio			
		0 lb. N	20 lb. N	40 lb. N	60 lb. N
1948	58·18	1·41	1·39	1·41	1·31
1949	77·79	1·50	1·53	1·62	1·65
1950	57·15	1·29	1·25	1·24	1·28
1951	41·93	2·04	2·04	1·96	2·06
Mean	58·299	1·56	1·55	1·56	1·58

It will be seen from Table VI, that in the year of normal rainfall (as it had been in the years 1948 and 1950), straw to grain ratio did not increase with the increasing doses of ammonium nitrate. But in the years of higher rainfall of 1949 (77·79 in.) straw to grain ratio increased with the increasing doses. In the year of low rainfall of 1951 (41·93 inches), the straw to grain ratio on the other hand was higher than that in the other years due to many sterile spikelets, but this ratio did not increase with the increasing doses.

(d) *Effect on soil due to the application of ammonium nitrate*

At the end of the experiment, soil was sampled from each sub-plot to the ploughed depth at random. Soils from the sub-plots of each of the nitrogen treatments and control were mixed thoroughly and finally 4 soil samples were prepared (passed through a 0·2 mm. sieve). In order to find out whether continuous application of ammonium nitrate produced any effect on the soil, these samples were chemically analysed. The results of analysis are given in Table VII.

TABLE VII

Analysis of soil under different doses of ammonium nitrate after carrying out the experiments during 1948-52

Doses per acre	N (per cent)	C (per cent)	Av. P_2O_5 (per cent)	*Ex*- Ca (m.e. per cent)	*Ex*. bases (m.e. per cent)	*p*H
0 lb. N	0·088	0·774	0·0040	14·5	20·6	6·9
20 lb. N	0·104	0·840	0·0058	14·8	20·8	6·9
40 lb. N	0·098	0·909	0·0058	15·2	21·5	6·7
60 lb. N	0·089	0·745	0·0058	14·3	20·8	6·8

The use of ammonium nitrate did not lower the *p*H value of the soil to any appreciable extent. The base status and lime status of the soil also remained practically unchanged. The nitrogen, carbon and available P_2O_5 content of the soil also behaved similarly. From the results of this field experiment, carried out for 4 years, it may be concluded that ammonium nitrate can be used for increasing the yield of paddy without any bad effect on the soil within a short period.

Summary

1. Effect of three doses of ammonium nitrate, i.e. 20, 40, and 60 lb. nitrogen per acre on the yield of *aman* paddy was observed in trials carried out for four years. It had been found that the yield of grain and straw increased significantly up to the dose of 40 lb. of nitrogen per acre.

2. Three 'times of application' of ammonium nitrate were compared. It appears that full dose at puddling was somewhat better than the full dose applied 4 weeks after transplantation. The other 'time of application', e.g. half at puddling and half 4 weeks after was not significantly different from either of the two as mentioned above.

3. Straw to grain ratio was almost independent of manurial treatments.

4. Nitrogen, carbon, available P_2O_5, exchangeable bases and exchangeable calcium content and *p*H value of the soil were not altered to any appreciable extent due to the treatment with ammonium nitrate during the 4 years period.

Acknowledgment

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STUDIES ON THE SURVIVAL OF PATHOGENS IN NIGHT-SOIL COMPOST

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(With two Text-Figures)

MUCH interest is now being evinced in compost production in most of the States in India. Several schemes for compost manufacture from refuse and human excreta have been launched and a vigorous campaign for popularising compost manure is carried on by the Agricultural Departments. As a part of the propaganda under the Grow More Food campaign slogans such as 'Wealth from Waste' have received wide publicity. All this may sound alright to create a demand for compost, but it will never fully succeed unless the related public health aspects are also properly integrated in this plan.

Ever since Hutchinson and Richards [1921] enunciated the scientific principles of converting wastes into good organic manure, several notable advances have taken place in this field. In 1939, Acharya developed the Bangalore method of composting in which refuse and human excreta are used for manufacture of compost and this method has been advocated by the Central and State Governments in the country for disposal of nightsoil and refuse in rural and municipal areas. From the public health point of view the practice of using nightsoil for production of manure raises an important question, viz. 'is there any public health risk involved in the production and use of nightsoil compost as manure?'

There are very few investigations relating to the hygienic aspects of nightsoil composting in public health literature. Papers published from Western countries do not contain any experimental data on the extent to which pathogens survive in nightsoil compost as this method is rarely practised in those regions. In the East, Winfield, Winslow and Scott [1939] conducted some studies on the microbiology and health aspects of nightsoil composting in China. Some observations on the destruction of helminthic ova in nightsoil composting were also reported by Scharff [1940] from Malaya and by Nichols and Gunewardhane [1939] from Ceylon. More recently Van Vuren [1949] and Blair [1951] have also reported work carried out on this problem in South Africa. No work has been done in India on the hygienic aspects of composting although this method of disposal of refuse and excreta is widely practised in different parts of the country. The present work was, therefore, undertaken at the instance of the Indian Council of Agricultural Research to fill the gap in our knowledge on this problem.

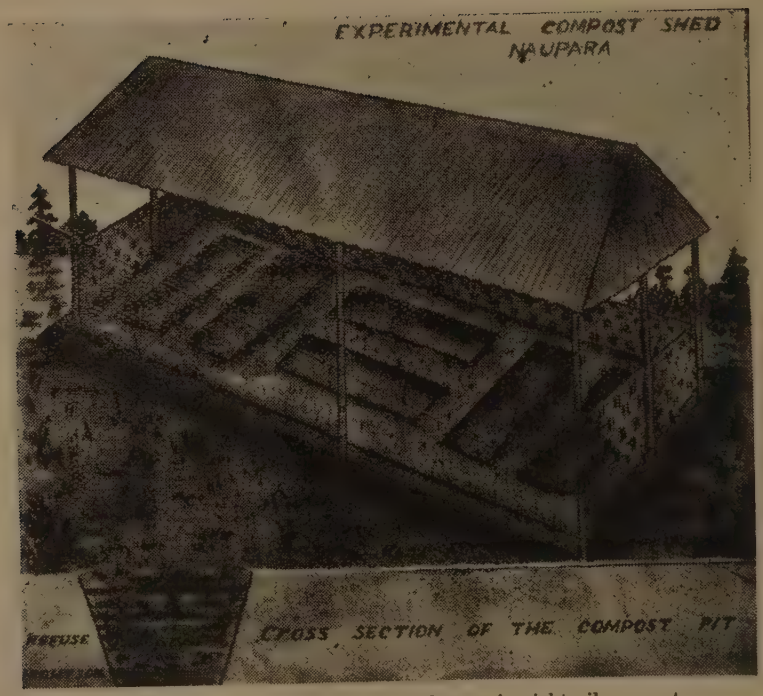
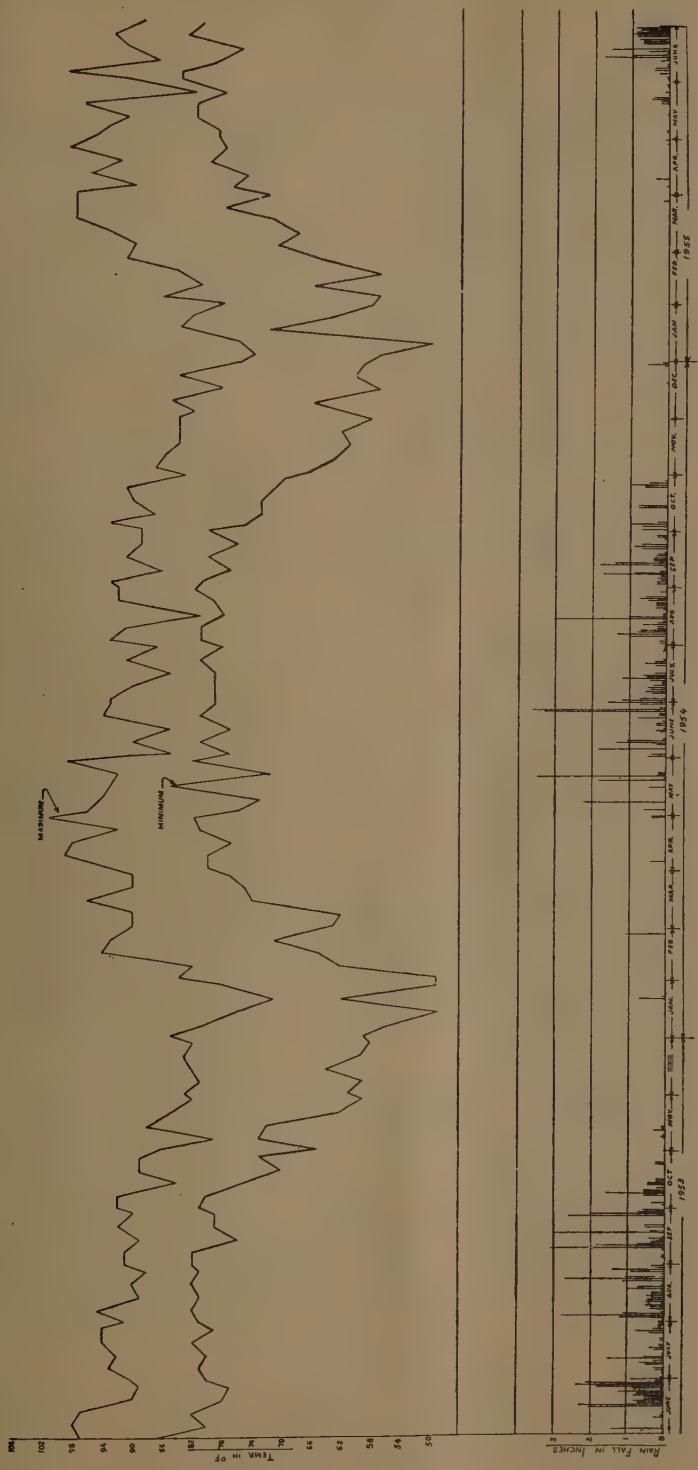


FIG. 1—Studies on the survival of pathogens in nightsoil compost



Rainfall and temperature conditions

The object of this investigation was to study the fate of pathogenic organisms of intestinal origin in the composting process and to determine the conditions under which nightsoil compost could be freed from the risk of infection. Data on the presence and viability of pathogens of the *Salmonella* and *Shigella* group of bacteria and the eggs of intestinal helminthic parasites such as, ascaris, hook-worm and trichuris were obtained by analysing compost samples from different parts of the country as well as from experimental compost pits maintained under controlled conditions. The results obtained indicate the degree of sanitary efficiency in composting as practised in different parts of the country and are also useful in assessing the hygienic risk involved in the production and utilisation of nightsoil compost for agricultural purposes.

Materials and Methods

For the purpose of this investigation representative samples of compost produced in different parts of the country were obtained from the State Agricultural Departments through the help of Dr Talati, Compost Development Officer of the Government of India.

For obtaining data under controlled conditions eight experimental compost pits were made in the trenching ground area in Naupara about seven miles from Calcutta, according to the Bangalore method of composting nightsoil and refuse as described by Acharya. A shed 50 ft.×20 ft. was provided to protect the compost pits from the rain. The shed was open on all sides and otherwise exposed to atmospheric conditions. Refuse and nightsoil used for making the compost were obtained from the daily collections of the Corporation of Calcutta. Representative samples of nightsoil material were also microscopically examined before filling in the pits (Table II). The experimental compost pits were of the following measurements—top 6 ft. 3 in.×3 ft. 3 in., bottom 6 ft. 1 in., depth 2 ft. City refuse and nightsoil were stacked in the pits in alternate layers of 6 in. and 2 in. respectively. In some of the pits refuse and nightsoil were mixed together in equal proportions of weight before filling in the pits. Two compost pits were also made in the open air in addition to those inside the shed. Top surface was covered with earth. Layout and cross-section of compost pits are given in Fig. 1.

All the compost pits were under the supervision of a Sanitary Inspector. A sweeper was stationed in Naupara to see that the pits were properly taken care of. A record of the daily weather conditions in the area during the period of experimentation was maintained (Fig. 2). The temperature of the compost was recorded once a week by inserting a special type of earth thermometer (Casella, London—Ranging up to 180°F.) to different depths. Samples were collected from these pits periodically for analysis. The sample collection was made by 2 in. augur taking care to see that a representative sample was obtained from the pits at different stages.

The samples obtained from different States in the country as well as those from the experimental compost pits were examined for the following items :

(*a*) Moisture, carbon and nitrogen ;

(*b*) Microscopical examination for the presence of ascaris, hook-worm and Trichuris eggs ;

(*c*) Viability of the ova examined in (*b*) ;

(*d*) Bacteriological examination of samples for *Salmonella* and *Shigella* group of organisms.

Carbon and nitrogen in the samples were determined by the methods described by Bhaskaran, *et al.* [1936]. The microscopical examination was carried out by the procedure described by Bhaskaran *et al.* [1956] for examination of sludge samples. 100 gm. of the compost sample was mixed up with 250 cc. of tap water and the suspension thus obtained was left at cold temperature for 24 hours. Next day salt was added to the mixture to saturate it. The suspension was then allowed to stand for three hours when all the ova came to float. The top layers of the suspension (about 50 cc.) were carefully pipetted and aliquot portions of the same were examined microscopically for enumeration of ova.

The following technique was used for the bacteriological examination of the compost samples :

Approximately 2 gm. of each sample was thoroughly shaken in 10 cc. of nutrient broth. After the heavier particles settled down at room temperature, the opalescent suspension was used as the inoculum. The solid media used for this purpose were (1) Desoxycholate-Citrate Agar (DCA) and (2) Wilson and Blair's Bismuth Sulphite medium. Kristensen's Brilliant agar was also used in the earlier part of the investigation, but was later discarded because this medium had no obvious advantages. 0·2 to 0·5 cc. of the inoculum was placed on each of the DCA or Wilson and Blair's solid medium contained in petri dishes (6 in. diameter) and spread over the whole surface. The plates were incubated at 37°C for 48 to 72 hours and examined for characteristic colonies of *Salmonella* and *Shigella* group of organisms. Kauffman's tetra-thionate broth was used as the routine enrichment medium for the *Salmonella* other than *S. typhosa*. About 2 gm. of compost was directly inoculated into the broth and the medium incubated for 36 to 48 hours. Loopfulls were then sub-cultured on the solid media mentioned above and examined as before. Suspected colonies were picked up and investigated further by the routine biochemical and serological tests.

The compost samples from Naupara were put up for culture on the same day as they were received. Out-station samples were stored at 0°C in the laboratory for periods up to two weeks before culturing. In all cases, no attempt was made to enumerate the total bacteria or to culture the samples anaerobically.

The above studies were carried out for a period of two years and the results obtained are presented in Tables I-V.

TABLE I

Description of compost samples collected from different places in India

State	Place	Material and Method used for composting	Method of sampling	Number of sample examined								Total
				Approximate age of samples in months								
				2	3	4	5	6	8	9	12	
Bombay	Trenching ground, Kirkee, Poona	Nightsoil: rubbish 1:1 by weight filled 3 ft. covered with 6 in.layer of earth	Drawn at random from 3 places upto 3 ft. depth, mixed and 2 samples of about ½ lb. collected		1	1		1	1		1	5
Uttar Pradesh	Lucknow Cantt. Board Trenching Ground	Nightsoil: Rubbish 1:1 by wt. filled 3 ft. covered with 10 in. layer of earth	Drawn at random from 8 points upto 3 ft. depth such 3 samples were ½ lb. collected	1		1		1	1	1	1	6
Hyderabad	Yousugada Trenching Ground	Nightsoil: Rubbish in alternate layer 1 ft. 6 in.; First and last rubbish 3 ft. deep covered with 3 in.-4 in. layer of earth	First one from 6 points 2nd from 4 points. Mixed thoroughly and ½ lb. collected	1		1		1	1	1	1	6
Mysore	Chellaghata Compost Yard	1:1 Nightsoil: Street rubbish Bangalore method pits of 2 ft. depth to a height of 3 ft. above ground level	Drawn at random from 8 points per trench upto 3 ft. Such 3 trenches were being sampled and ½ lb. was collected		1	1		1	1		1	5
East Punjab	Jullunder City	Nightsoil, street and house hold sweepings added Gammexane covered with 1 in.-2 in. layer of earth	About a seer of sample taken from 4 trenches and representative taken	2	1	2	1	1				7

TABLE II

Analysis of nightsoil used in different compost pits in Naupara Trenching Ground

Pit No.	No. of ova in 100 gm. of the sample			
	Ascaris	Trichuris	Hookworm	Amoebic cysts
1	1,500	..	500	*Nil*
2	3.000	..	300	*Nil*
3	2,000	..	*Nil*	Present (about 20)
4	1,600	200	100	*Nil*
5	3,200	200	*Nil*	*Nil*
6	2,500	300	*Nil*	*Nil*
7	1,900	300	*Nil*	*Nil*
8	300	*Nil*	*Nil*	*Nil*

TABLE III

Results of examination of compost samples collected from the experimental compost pits in Naupara

Method of composting	Pit No.	Season	Number of Ascaris eggs in 100 gm. of compost sample					
			Age of samples in months					
			1	2	3	4	5	6
Refuse and nightsoil filled in pit in alternate layer of 6 in. and 2 in. respectively	I	2-3-54 to 6-9-54		600 (5)	80 —*nil*—		60 —*nil*—	—*nil*—
	II	2-3-54 to 6-9-54		500 (5)	40 —*nil*—		40 —*nil*—	20 —*nil*—
	III	6-5-54 to 6-11-54	100 (6)		40 —*nil*—	20 —*nil*—		
	IV	31-5-54 to 6-11-54		60 —*nil*—	40 —*nil*—			
	Under the shed							
Refuse and nightsoil mixed in proportion of 1 : 1 by weight and filled in pits	V	20-11-54 to 23-3-55	200 (3)	80 —*nil*—	20 —*nil*—	—*nil*—		
	VI*	20-11-54 to 23-3-55	240 (2)	60 —*nil*—	—*nil*—	—*nil*—		
	In the open							
	VII	25-11-54 to 25-3-55	300 (4)	100 —*nil*—	—*nil*—	—*nil*—		
	VIII*	25-11-54 to 25-3-55	300 (3)	120 —*nil*—	40 —*nil*—	—*nil*—		

*Results in brackets refer to percentage viability of the eggs isolated in samples.

TABLE IV

Results of examination of compost samples from different States

State from where samples collected	No. of Ascaris ova found in 100 gm. of compost samples collected after different period in month								No. of ova found in 100 gm. of compost		Pathogenic bacteria in 5 gm. of compost	
	2 months	3 months	4 months	5 months	6 months	8 months	10 months	12 months	Hook-worm	Tri-churis	Salmo-nella	Shigella
Bangalore	*nil*		50			10		10 20	*nil*	*nil*	Absent	Absent
Bombay	40(0) 240(4)		100(0) 60(0)		40(0)	10		*nil*	*nil*	*nil*	do.	do.
Hyderabad	200(0)		200(6)		30(0)	80(0)		*nil*	*nil*	*nil*	do.	do.
Lucknow	20(0) 40(4)		*nil*		200(0) 240(8)	40		*nil*	*nil*	20 in sample No. 2 (6 months old)	do.	do.
Punjab	20(0)		*nil*		*nil*	*nil*		*nil*	*nil*	*nil*	do.	do.

TABLE V

Chemical analysis of compost samples

(a) *Samples from different States*

Place of collection	Percentage moisture					Percentage nitrogen					Carbon/nitrogen				
	Age in months					Age in months					Age in months				
	2	4	6	8	12	2	4	6	8	12	2	4	6	8	12
Lucknow	8·7	5·4	15·1	1·3	3·3	0·61	0·83	0·96	1·12	1·18	7·0	7·0	6·8	6·0	6·7
Hyderabad	11·1	4·9	2·4	2·6	1·9	0·46	0·25	0·34	0·91	0·35	6·3	6·8	5·8	6·7	7·6
Bangalore	6·8	6·3	..	5·4	3·9	0·39	0·63	..	0·82	1·02	6·7	6·8	..	7·8	6·9
Poona	15·5	37·1	30·6	14·3	3·2	0·93	1·26	1·53	0·82	1·08	6·4	5·7	4·8	8·2	6·9
Ludhiana (Punjab)	4·4	11·9	4·0	..	..	0·52	0·63	0·97	..	..	6·5	6·8	6·4	..	..

(b) *Samples from experimental compost heaps*

Naupara	Pit No.	Percentage moisture			Percentage nitrogen			Carbon/nitrogen		
		Age in months			Age in months			Age in months		
		2	3	4	2	3	4	2	3	4
	I	29·4	33·5	..	0·68	1·18	..	4·9	7·3	..
	II	32·4	35·3	..	0·72	1·14	..	5·6	7·1	..
	III	..	7·9	13·2	..	0·97	1·20	..	7·5	7·4
	IV	16·8	12·7	15·0	0·66	1·10	1·19	7·2	7·0	7·6
	V	27·1	17·6	8·2	0·50	0·88	1·29	7·2	7·3	6·5
	VI	21·1	12·5	7·9	0·64	0·94	1·16	6·4	6·9	7·0
	VII	25·6	28·5	3·7	0·83	0·86	1·13	6·3	6·7	7·0
	VIII	28·3	15·1	5·3	0·86	0·88	1·10	6·9	6·5	8·1

Data presented above show that if the composting is carried out under good supervision and the pits are maintained properly all the pathogenic bacteria and parasitic ova are destroyed during the process within a period of three months. The results obtained for samples collected from Naupara trenching ground show that even within one month both the number and viability of the helminthic ova are rapidly reduced. The temperature inside the pits rose up to 104°F and persisted for a period of 10 to 15 days when the outside temperature ranged from 78°–83°F. This rising temperature within the compost pits which continues for several days and the maintenance of anaerobic environment inside the compost leads to the rapid destruction of the pathogens in the nightsoil. In the Naupara experiments, the location of the pits within the shed does not seem to have made any significant difference (Table III). However, the results of the samples collected from different States were somewhat irregular. In a few instances, even six month old samples showed the presence of a few eggs some of which were viable.

The present studies show that the sanitary efficiency of composting is largely governed by adequate care and control in the making of compost pits and proper sanitary supervision during maturity, so that suitable temperatures are developed within the compost pits and persist for a period of at least 10 days. If composting is to be recommended as a method of nightsoil disposal, the public health authorities should see that the required care and supervision are exercised at each stage of the composting process.

Chemical analysis of the compost obtained with nightsoil and town refuse shows that the product forms a well-digested manure which has potential agricultural value, having about 1 per cent nitrogen and C/N ratio of 6 to 8

Conclusion

The results presented on the survival of pathogenic organisms in the Bangalore method of composting of human excreta and town refuse show that all the pathogens present in excreta are completely destroyed under this method of composting. Both the number and viability of helminthic ova such as ascaris and hook-worm decrease rapidly within the first one month and they are completely eliminated in the course of three months. Bacteriologically also, all the samples were uniformly negative.

Another important observation arising out of these studies is that composting of nightsoil and refuse can be satisfactorily used for hygienic disposal of human excreta provided the composting operations are carried out under controlled supervision. The responsibility for such supervision should rest with the health departments of the different regions. If the health departments of the States arrange for regular supervision of the composting operations so as to ensure that the compost pits are properly made up and are fully ripe before the material is taken out of the pits and used as manure, then there is very little hygienic risk involved in the use and handling of compost for agricultural purpose.

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Grateful acknowledgement is made to Dr. Talati, Compost Development Officer, Government of India for arranging to procure compost samples from different parts of the country. The authors express thanks to the Director, All India Institute of Hygiene and Public Health, Calcutta, for the facilities provided to carry out this investigation and to Prof. F. K. Erickson, Professor of Sanitary Engineering for helpful suggestions in the course of this work.

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RELATIONSHIP BETWEEN FODDER AND SEED YIELDS OF BERSEEM AND RESPONSES TO PHOSPHATE LEVELS

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(With 9 Text-Figures)

An experiment was started in 1941-42 with a view to studying the effect of low doses of P_2O_5 applied in the form of superphosphate and farmyard manure and their certain combinations on berseem and after-effects on wheat. The results obtained in the first cycle [Parr and Bose, 1944 ; 1947] in which berseem was manured consecutively for three years, followed by unmanured wheat for three succeeding years, showed that P_2O_5 had significant effect on the yield of berseem and application of superphosphate generally gave higher yields of fodder than farmyard manure, but the differences were mostly not significant. With wheat, significant increases in yield over the control were obtained wherever the dose of P_2O_5 applied to berseem was 64 lb. per acre either as superphosphate alone or superphosphate in combination with farmyard manure.

The second cycle of the rotation was started in 1948-49 and a summary of yield results obtained with three crops of berseem grown from 1948-49 to 1950-51 together with studies on the effect of phosphate fertilisation on the nodulation of berseem and nitrogen and available P_2O_5 contents of the soil has been dealt with separately [Sen and Bains, 1955].

In this article a critical examination of the yield data of the berseem fodder and seed obtained in the second cycle has been made with special reference to the study of :

(i) the relationship between yields of fodder and seed,

(ii) responses due to different doses for four classes of fertiliser treatments, and

(iii) relative performance of four classes of fertilizers and the interaction of doses with classes of fertilizers

Experimental

The manurial treatments included in the field trial are given below :

A. Farmyard manure at 16 lb. P_2O_5 per acre

B. Farmyard manure at 32 lb. P_2O_5 per acre

C. Farmyard manure at 64 lb. P_2O_5 per acre

D. Superphosphate at 16 lb. P_2O_5 per acre

E. Superphosphate at 32 lb. P_2O_5 per acre

F. Superphosphate at 64 lb. P_2O_5 per acre

G. Superphosphate at 8 lb. P_2O_5 + Farmyard manure at 8 lb. P_2O_5 per acre
H. Superphosphate at 8 lb. P_2O_5 + Farmyard manure at 24 lb. P_2O_5 per acre
I. Superphosphate at 8 lb. P_2O_5 + Farmyard manure at 56 lb. P_2O_5 per acre
J. Farmyard manure at 8 lb. P_2O_5 + Superphosphate at 24 lb. P_2O_5 per acre
K. Farmyard manure at 8 lb. P_2O_5 + Superphosphate at 56 lb. P_2O_5 per acre
L. No manure—'control'

The experiment was conducted on the same site for three years.

The experiment was laid out in a randomised block design with six replications. The plot size was 1/46 acre approximately. The agronomic details of the experiment are given in Table I.

TABLE I

Details of agricultural operations

Date	1948-49	1949-50	1950-51
Sowing	11-11-48	26-11-49	4-11-50
Manuring	9-11-48	16-11-49	1-11-50
Harvesting fodder (i)	19-1-49	2-3-50	25-1-51
.. (ii)	11-3-49	4-4-50	4-3-51
.. (iii)	11-4-49	25-4-50	1-4-51
No. of irrigations	15	12	13

I. RELATIONSHIP BETWEEN YIELD OF FODDER AND YIELD OF SEED

1. *Testing linearity of relationship between yields of fodder and seed*

In the study of relationship between the yields of seed and fodder, the analysis has been made to get information on the linearity or otherwise of the regression line of yield of seed (y) upon the yield of fodder (x) for the 12 treatment means. The procedure adopted was to split up the 11 d.f. for the treatment sum of squares of y into 1 for linear regression and the remainder 10 for deviation from linearity and testing the variance due to the latter against the reduced error variance with 54 d.f.

The essential features of the analysis are given in Table II.

TABLE II

Analysis of covariance and reduced variance

	DF	SS(x)	SP(xy)	SS(y)	DF	E (y-Y)°	Reduced variance	F
				1948-49				
Treatments	11	1359877·15	13786·6458	177·8646	10	38·0934	3·8093	1·59
Error	55	484103·10	2592·7292	142·9896	54	129·1036	2·3908	
Treatments + error	66	1843980·25	16379·3750	320·8542	65	175·3624		
Difference between b(T) and b (E)					1	8·1654	8·1654	3·42
				1949-50				
Treatments	11	3167625·04	15652·4354	85·9609	10	8·6163	0·8616	2·10*
Error	55	732286·38	1612·4446	25·6712	54	22·1207	0·4096	
Treatments + error	66	3899911·42	17264·8800	111·6321	65	35·2006		
Difference between b (T) and b (E)					1	4·4636	4·4636	10·90**
				1950-51				
Treatments	11	2773560·44	23157·2528	212·7631	10	19·4165	1·9417	1·77
Error	55	449935·06	1827·5464	66·5441	54	59·1212	1·0948	
Treatments + error	66	3223495·50	24984·7992	279·3074	65	85·6542		
Difference between b (T) and b (E)					1	7·1165	7·1165	6·50**
				Average over three years				
Treatments	11	6765837·32	51432·8291	420·9372	10	29·9530	2·9953	2·22*
Error	55	1158482·68	4426·0809	89·7017	54	72·7915	1·3480	
Treatments + error	66	7924320·00	55858·9100	510·6389	65	116·8869		
Difference between b (T) and b (E)					1	14·1424	14·1424	10·49

NOTE.—(i) b (T) and b (E) denote regression coefficients for treatments and error respectively.

(ii) * and ** denote significance at 5 and 1 per cent respectively.

It is seen from the results shown in Table II that a considerable amount of variation in seed yield due to treatments has been accounted for by the variation in fodder yield so much so that the remaining variance of seed yield due to treatments became nonsignificant in two cases and just significant at 5 per cent level in the other cases. This shows that the treatment means for x and y are closely related so that the manurial treatments which enhance the yield of fodder also enhance the yield of seed.

2. *Regression and correlation coefficients*

Table III gives the regression and correlation coefficients and other constants for the three years for treatments and error terms of Table II. The linear regression equation between y and x may be put in the form $y = a + bx$.

TABLE III

Regression and correlation coefficients (y, a and x in lb. per plot)

Year	a(T)	b(T)	±S. E b(T)	r(T)	a(E)	b(E)	±S. E b(E)	R(E)
1948-49	0·9713	0·010138***	0·001674	0·8865***	3·3533	0·005356*I	0·002222	0·3116*I
1949-50	—0·3734	0·004941***	0·000522	0·9486***	1·1926	0·002202***	0·000748	0·3719***
1950-51	1·6219	0·008349***	0·000837	0·9533***	3·3724	0·004062*I	0·001560	0·3340*I
Average over 3 years	0·7558	0·007602***	0·000665	0·9638***	2·8863	0·003277***	0·001155	0·2517***

NOTE.—(i) (T) and (E) refer to treatment and error respectively.

(ii) *I indicates significance at 2 per cent level and ***indicates significance at 0·1 per cent level.

It is seen from Table III that the regression and correlation coefficients are highly significant.

This shows that the yields of seed and fodder are highly associated, i.e. increase of one is accompanied by a corresponding increase of the other.

The line corresponding to 1 DF in Table II is for the difference between the regression coefficients b (T) and b (E). The 'F' value of the reduced variance corresponding to this one degree of freedom is highly significant for the second two years and also for the average over the three years, while it is not significant for the first year. The regression coefficients in Table III show that the average increase in weight of seed for unit increase in weight of fodder is generally more (nearly twice) in presence of treatment effects than that obtained after the removal of block and treatment effects.

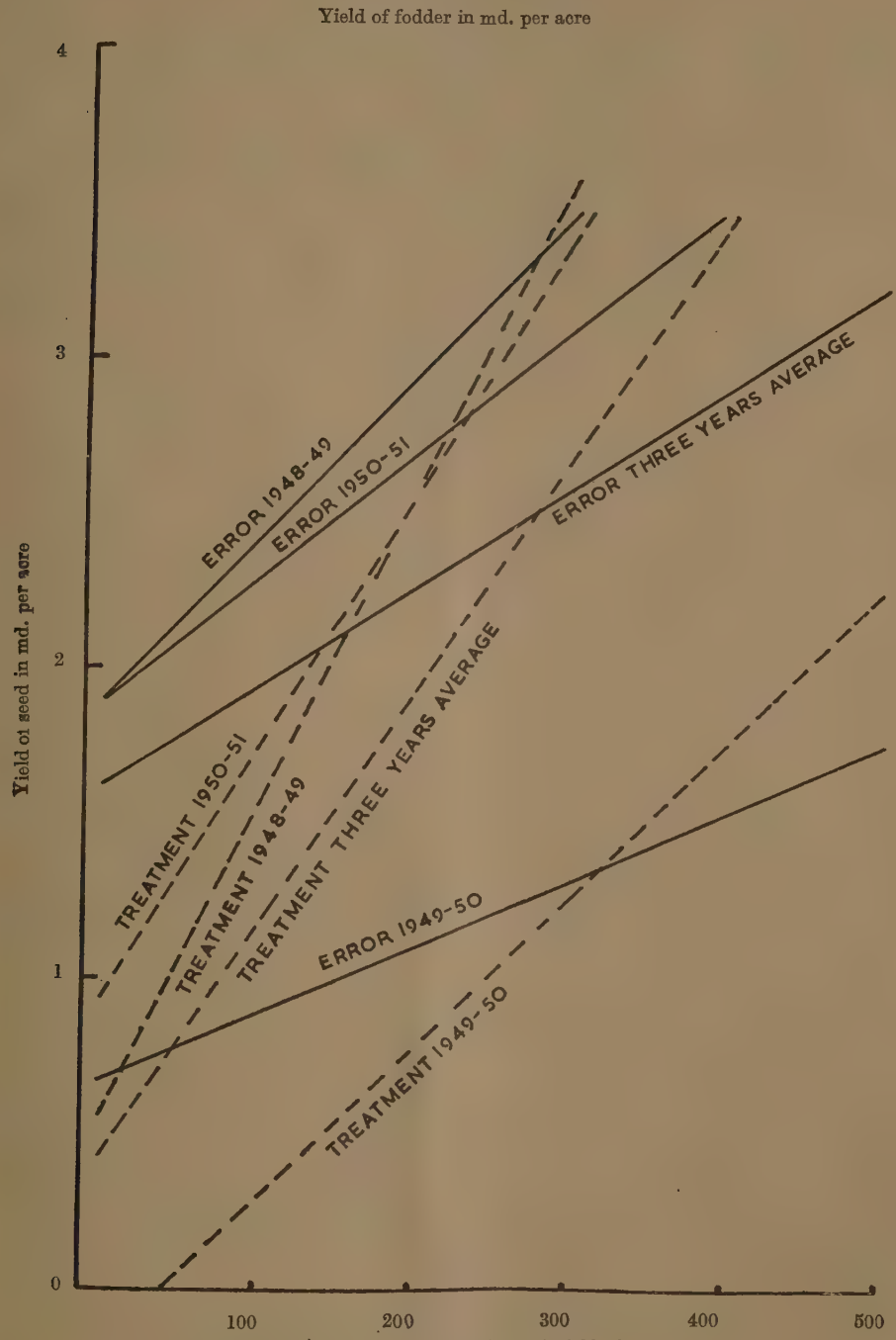

FIG. 1—Relationship between yield of fodder and yield of seed in md. per acre

Fig. I gives the relationship $y = a + bx$ fitted in presence of the treatment effects (dotted lines) and also the straight line obtained from the error terms (continuous lines).

Tables IV and V give the analysis of variance to test the differences between b (E) for the three years and b (T) for the same period respectively.

TABLE IV

Testing differences b (E)

Sources	SS	DF	MSS	'F'
Separate regressions	24·8596	3		
Joint regression	21·8407	1		
Difference between b (E)s	3·0189	2	1·5094	1·16
Error	210·3156	162	1·2984	

TABLE V

Testing difference b (T)

Sources	SS	DF	MSS	'F'
Separate regression	410·4623	3		
Joint regression	378·9002	1		
Difference between b (T) s	31·5621	2	15·7810	7·16***
Error	66·1262	30	2·2042	

Tables IV and V show that while differences between b (T)s are highly significant, these are not so between b (E)s. However, the orders in magnitude of b (T)s and b (E)s are the same, namely, 1948-49, 1949-50 1950-51.

Denoting the years 1948-49, 1949-50, 1950-51 by I, II and III respectively we have, for the differences between the b (T)s, the standard errors of the differences as given in Table VI.

TABLE VI

Testing regression coefficients between the years for treatments

Due to	Difference=d	SE of d	t
I—II	0·005197	±0·001522	3·41**
I—III	0·001789	±0·001554	1·15
II—III	—0·003408	±0·001221	2·79**

Table VI indicates that while b_I and b_{III} are significantly greater than b_{II}, the difference between them is not significant.

The third cutting of berseem in 1949-50 got delayed which enhanced the yield of green fodder and had an adverse effect on the seed yield.

In order to see if the degree of relationship between y and x was the same for all the three years, the correlation coefficients for E and T were tested for homogeneity. The values of χ^2 for E and T which are 0·12 and 1·03 respectively are not significant. This shows that the degree of relationship between x and y does not materially change from year to year, either when treatment means are considered or when the effects due to block and treatments eliminated.

II. Responses due to Different Doses for four classes of Fertilizer Treatments

1. As described earlier, the yield of green fodder (x) and seed (y) are highly correlated. However, it is found that the nature of response in x and y for different qualities over the levels of manurial treatments are not very similar. The present section deals essentially with responses to superphosphate and farmyard manure in varying doses. It may be pointed out that no attempt has been made here to find the best response curve, as this cannot be done when the number of levels of each treatment are just three or four. Therefore, it was decided to see if the response was linear or not linear. In the case of four doses, quadratic response too has been included.

For purpose of brevity the doses have been denoted as :
Superphosphate treatment by S1
Farmyard manure by F1
Superphosphate + 8 lb. P_2O_5 as farmyard manure by S2
Farmyard manure +8 lb. P_2O_5 as superphosphate by F2

2. *Nature of response curves for the four classes of treatments*

Response curves were studied up to 2nd degree by fitting orthogonal polynomials to the total yields of six blocks for S1 and F1 treatments, the doses being 0, 16 lb., 32 lb. and 64 lb. For S2 and F2 treatments, only linearity or deviation from linearity was studied as there were only three doses, viz. 8 lb. P_2O_5 (farmyard manure) +8 lb. P_2O_5 (superphosphate) ; 8 lb. P_2O_5 (farmyard manure) + 24 lb. P_2O_5 (superphosphate) ; and 8 lb. P_2O_5 (farmyard manure) + 56 lb. P_2O_5 (superphosphate) for S2 and 8 lb. P_2O_5 (superphosphate) + 8 lb. P_2O_5 (farmyard manure) ; 8 lb. P_2O_5 (superphosphate) + 24 lb. P_2O_5 (farmyard manure) and 8 lb. P_2O_5 (superphosphate) + 56 lb. P_2O_5 (farmyard manure) for F2.

Fig. I gives the relationship $y = a + bx$ fitted in presence of the treatment effects (dotted lines) and also the straight line obtained from the error terms (continuous lines).

Tables IV and V give the analysis of variance to test the differences between b (E) for the three years and b (T) for the same period respectively.

TABLE IV

Testing differences b (E)

Sources	SS	DF	MSS	'F'
Separate regressions	24·8596	3		
Joint regression	21·8407	1		
Difference between b (E)s	3·0189	2	1·5094	1·16
Error	210·3456	162	1·2984	

TABLE V

Testing difference b (T)

Sources	SS	DF	MSS	'F'
Separate regression	410·4623	3		
Joint regression	378·9002	1		
Difference between b (T) s	31·5621	2	15·7810	7·16***
Error	66·1262	30	2·2042	

Tables IV and V show that while differences between b (T)s are highly significant, these are not so between b (E)s. However, the orders in magnitude of b (T)s and b (E)s are the same, namely, 1948-49, 1949-50 1950-51.

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Table VI indicates that while b_I and b_{III} are significantly greater than b_{II}, the difference between them is not significant.

The third cutting of berseem in 1949-50 got delayed which enhanced the yield of green fodder and had an adverse effect on the seed yield.

In order to see if the degree of relationship between y and x was the same for all the three years, the correlation coefficients for E and T were tested for homogeneity. The values of χ^2 for E and T which are 0·12 and 1·03 respectively are not significant. This shows that the degree of relationship between x and y does not materially change from year to year, either when treatment means are considered or when the effects due to block and treatments eliminated.

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1. As described earlier, the yield of green fodder (x) and seed (y) are highly correlated. However, it is found that the nature of response in x and y for different qualities over the levels of manurial treatments are not very similar. The present section deals essentially with responses to superphosphate and farmyard manure in varying doses. It may be pointed out that no attempt has been made here to find the best response curve, as this cannot be done when the number of levels of each treatment are just three or four. Therefore, it was decided to see if the response was linear or not linear. In the case of four doses, quadratic response too has been included.

For purpose of brevity the doses have been denoted as :
Superphosphate treatment by S1
Farmyard manure by F1
Superphosphate + 8 lb. P_2O_5 as farmyard manure by S2
Farmyard manure +8 lb. P_2O_5 as superphosphate by F2

2. *Nature of response curves for the four classes of treatments*

Response curves were studied up to 2nd degree by fitting orthogonal polynomials to the total yields of six blocks for S1 and F1 treatments, the doses being 0, 16 lb., 32 lb. and 64 lb. For S2 and F2 treatments, only linearity or deviation from linearity was studied as there were only three doses, viz. 8 lb. P_2O_5 (farmyard manure) +8 lb. P_2O_5 (superphosphate) ; 8 lb. P_2O_5 (farmyard manure) + 24 lb. P_2O_5 (superphosphate) ; and 8 lb. P_2O_5 (farmyard manure) + 56 lb. P_2O_5 (superphosphate) for S2 and 8 lb. P_2O_5 (superphosphate) + 8 lb. P_2O_5 (farmyard manure) ; 8 lb. P_2O_5 (superphosphate) + 24 lb. P_2O_5 (farmyard manure) and 8 lb. P_2O_5 (superphosphate) + 56 lb. P_2O_5 (farmyard manure) for F2.

In every case the reduction in variance after each fitting was tested against error variance with 55 degrees of freedom.

3. *Responses in fodder*

Table VII regarding the yield of green fodder gives the various steps leading to the tests of departure from linearity, etc. for the different treatments. All quantities are based upon lb. per plot. W is expressed in the form $W = b_0 + b_1 Z_1 + b_2 Z_2 + \ldots\ldots\ldots\ldots$, where Z_r is the orthogonal polynomial of rth degree and b_r the corresponding regression coefficient. The relevent tables of the orthogonal polynomials used are given in the Appendix (iii); the procedure of fitting these is essentially the same as that for fitting the orthogonal polynomials as given by Fisher and Yates [1943].

TABLE VII

Testing the degree of response (fodder)

Class	Source	SS	DF	MSS	'F'	Nature of response
			(1948-49)			
S1	R (b_1)	18231·12	2	9115·56	1·04	Linear
	R (b_2)	91·69	1	91·69	0·01	
F1	R (b_1)	46777·03	2	23388·51	2·66	Linear
	R (b_2)	11094·60	1	11094·60	1·26	
S2	R (b_1)	344·05	1	344·05	0·39	Linear
F2	R (b_1)	1810·71	1	1810·71	0·21	Linear
Error		484103·10	55	8810·87	—	
			(1949-50)			
S1	R (b_1)	160473·36	2	80236·68	6·02	Quadratic
	R (b_2)	787·64	1	787·64	0·06	
F1	R (b_1)	31327·43	2	15663·71	1·18	Linear
	R (b_2)	17003·79	1	17003·79	1·28	
S2	R (b_1)	95344·05	1	95344·05	7·16	Non-linear
F2	R (b_1)	8·05	1	8·05	0·00	Linear
Error		732286·38	55	13314·30	—	
			(6950-51)			
S1	R (b_1)	100538·03	2	50269·01	6·14	Quadratic
	R (b_2)	465·02	1	465·02	0·06	
F1	R (b_1)	18192·07	2	9096·03	1·11	Linear
	R (b_2)	9146·74	1	9146·74	1·12	
S2	R (b_1)	51356·30	1	51356·30	6·28	Non-linear
F2	R (b_1)	640·76	1	640·76	0·08	Linear
Error		449935·06	55	8180·64	—	
			(Average over 3 years)			
S1	R (b_1)	242360·48	2	121180·24	5·75	Quadratic
	R (b_2)	1168·40	1	1168·40	0·06	
F1	R (b_1)	90922·47	2	4561·24	2·16	Linear
	R (b_2)	36601·60	1	36601·60	1·74	
S2	R (b_1)	102285·43	1	102285·43	4·86	Non-linear
F2	R (b_1)	1666·29	1	1666·29	0·08	Linear
Error		1158482·68	55	21063·32	—	

NOTE.—R (b_1) and R (b_2) are the residuals after fitting the linear and quadratic terms respectively.

A study of Table VII reveals the following :

(i) In 1948-49 responses in all the classes are linear.

(ii) In 1949-50, 1950-51 and for the average over three years, F1 and F2 gave linear responses, while S1 gave quadratic and S2 non-linear response. It is thus seen that response due to application of farmyard manure is linear for all three years while that due to superphosphate is quadratic for the second two years and also for the average over the three years, it being linear for the first year. (It may be noted that in 1948-49 the reduction in mean SS due to quadratic fitting over the linear fitting is considerable, showing thereby that the quadratic fit is better than he linear fit.)

It may, therefore, be concluded that the response due to application of farmyard manure is linear while that due to superphosphate is quadratic.

Figs. 2, 3, 4 and 5 give the response curves of fodder for the two doses of F1 and S1. Response curves for superphosphate and farmyard manure are given by continuous and dotted lines respectively.

4. *Response in seed*

TABLE VIII

Testing the degree of response (seed)

Class	Source	SS	DF	MSS	'F'	Nature of response
			(1948-49)			
S1	R (b_1)	23·7661	2	11·8830	4·57	Quadratic
	R (b_2)	3·6751	1	3·6751	1·41	
F1	R (b_1)	13·2161	2	6·6080	2·54	Linear
	R (b_2)	2·7690	1	2·7690	1·41	
S2	R (b_1)	1·3757	1	1·3757	0·53	Linear
F2	R (b_1)	12·3814	1	12·3814	4·76	Non-linear
Error		142·9896	55	2·5998		
			(1949-50)			
S1	R (b_1)	7·4384	2	3·7192	7·97	Quadratic
	R (b_2)	1·7608	1	1·7608	3·77	
F1	R (b_1)	4·5337	2	2·2669	4·86	Quadratic
	R (b_2)	1·4948	1	1·4948	3·02	
S2	R (b_1)	8·1906	1	8·1906	17·55	Non-Linear
F2	R (b_1)	1·5067	1	1·5067	3·23	Linear
Error		25·6712	55	0·4667		
			(1950-51)			
S1	R (b_1)	24·5641	2	12·2821	10·15	Quadratic
	R (b_2)	1·9789	1	1·9789	1·64	
F1	R (b_1)	6·6276	2	3·3138	2·74	Linear
	R (b_2)	0·3551	1	0·3551	0·29	
S2	R (b_1)	2·3802	1	2·3802	1·97	Linear
F2	R (b_1)	0·3963	1	0·3963	0·33	Linear
Error		66·5443	55	1·2099		
			(Average over three years)			
S1	R (b_1)	45·2107	2	22·6053	13·86	Quadratic
	R (b_2)	0·2223	1	0·2223	0·14	
F1	R (b_1)	20·3989	2	10·1995	6·25	Quadratic
	R (b_2)	1·7492	1	1·7492	1·07	
S2	R (b_1)	3·4815	1	3·4815	2·13	Linear
F2	R (b_1)	2·8438	1	2·8438	1·74	Linear
Error		89·7017	55	1·6309		

NOTE.—R (b_1) and R (b_2) are the residuals after fitting linear and quadratic terms respectively.

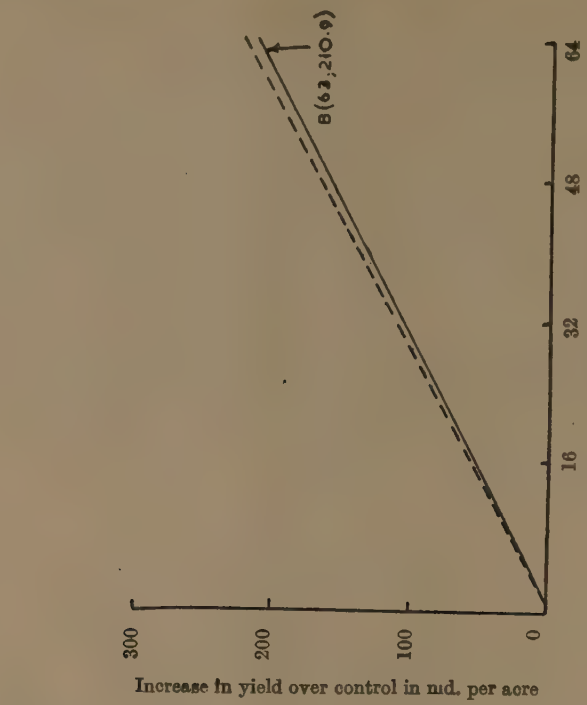

FIG. 3.—Response to superphosphate and F. Y. M.; fodder 1949-50

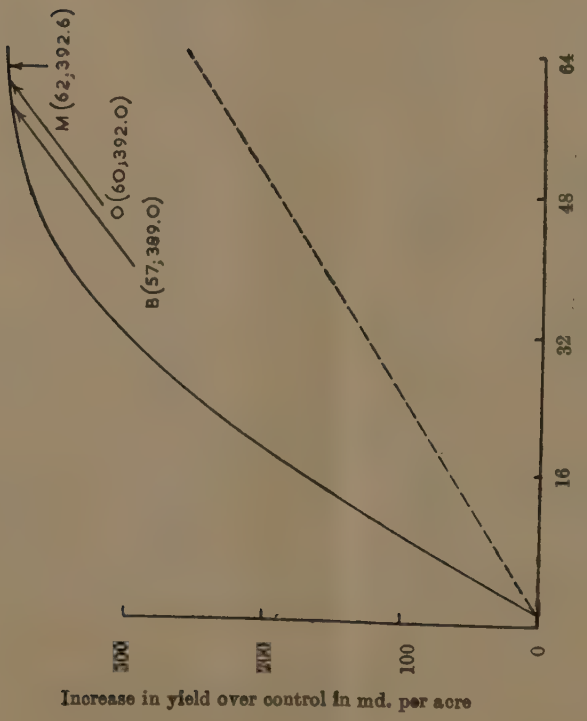

FIG. 2.—Response to superphosphate and F. Y. M.; fodder 1948-49

FIG. 4.—Response to superphosphate and F. Y. M.; fodder 1950-51

FIG. 5.—Response to superphosphate and F. Y. M.; fodder-average for 3 years

The following are revealed from a study of Table VIII :

(i) Responses due to S1 are quadratic for all the three years and also for the average over the three years.

(ii) Responses due to F1 are linear for first and third years, but quadratic for the second year and the average over the three years.

(iii) Responses due to S2 are linear for first, third and the average over the three years, but non-linear for the second year.

(iv) Responses due to F2 are linear for second, third and the average over the three years, but non-linear for the first year.

It will be observed that in five cases out of eight where varying doses of superphosphate have been applied, the response is quadratic or non-linear, and in five cases out of eight where farmyard manure has been applied in varying doses, the response is linear.

It is thus seen that the tendency in response of seed is similar to that of fodder though not so marked.

Figs. 6, 7, 8 and 9 give the response curves of seed for the two classes S1 and F1.

5. *Maximum, optimum and best responses*

In Table IX are given the yield equations as $Y = a + bx + cx^2$, where Y is the yield in maunds per acre for a dose of x lb. of fertilizer or manure per acre. Values of maximum yields, maximum response with corresponding dose of manure and optimum response with corresponding dose are also given. The optimum dose has been calculated by the formula :

$$X_{op} = \frac{M\text{-}b}{2c},$$

where $M = q/p$, q and p being the cost of unit x and price of unit y respectively. The cost of superphosphate was recorded at Rs. 17 per maund and that of farmyard manure at Rs. 0·2 per maund. The prices of seed and fodder were Rs. 75 and Re. 1 per maund respectively. P_2O_5 present in superphosphate and farmyard manure was 40 per cent and 0·59 per cent respectively.

On these bases, p worked out as 75 for seed ; q as 0·4855 and 0·5165 for farmyard manure and superphosphate respectively and M as 0·006474 for farmyard manure—seed, 0·4855 for farmyard manure—fodder, 0·006886 for superphosphate—seed and 0·5165 for superphosphate—fodder.

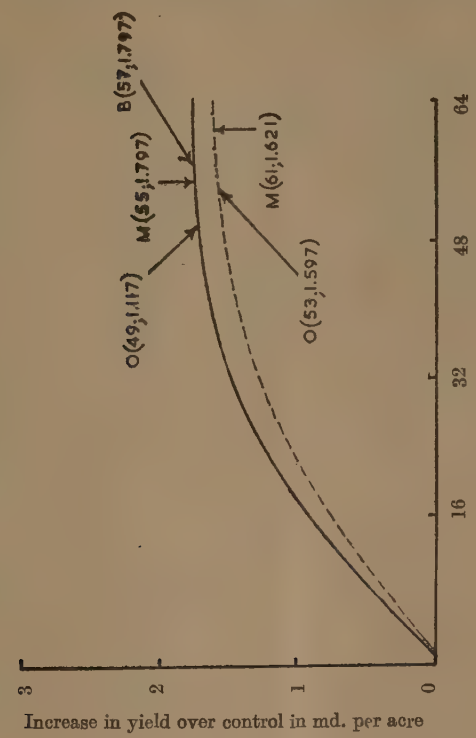

FIG. 7.—Response to superphosphate and F. Y. M.; seed 1949.

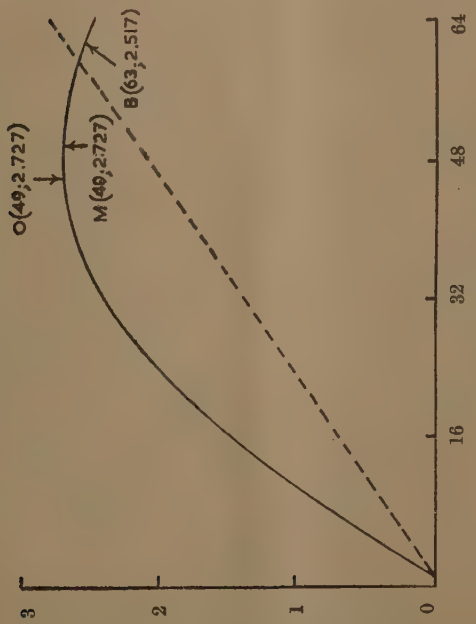

FIG. 6.—Response to superphosphate and F. Y. M.; seed 1948-49

FIG. 9.—Response to superphosphate and F. Y. M.; seed-average for 3 years

FIG. 8.—Response to superphosphate and F. Y. M.; seed 1950-51

TABLE IX

Yield equations, maximum, optimum and best responses to superphosphate and farmyard manure

Seed or fodder	Super or FYM	Yield equation	Maximum response	Optimum response	Best response
		(1948-49)			
Seed	Super	$Y=1{\cdot}2517+0{\cdot}1110x$ $-0{\cdot}001129X^2$	2·727(49)	2·717(46)	2·517(63)
	FYM	$Y=1{\cdot}6852+0{\cdot}04381x$	—	—	—
Fodder	Super	$Y=145{\cdot}72+3{\cdot}3537x$	—	—	210·9(63)
	FYM	$Y=145{\cdot}20+3{\cdot}4863x$			
		(1949-50)			
Seed	Super	$Y=0{\cdot}1988+0{\cdot}0657x$ $-0{\cdot}000600x^2$	1·797(55)	1·777(49)	1·797(57)
	FYM	$Y=0{\cdot}0321+0{\cdot}0534x$ $-0{\cdot}000439x^2$	1·621(61)	1·597(53)	0·618(108)
Fodder	Super	$Y=96{\cdot}69+12-{\cdot}5748x$ $-0{\cdot}1007x^2$	392·6(62)	392·0(60)	389·0(57)
	FYM	$Y=101{\cdot}77+4{\cdot}0914x$	—	—	444·1(108)
		(1950-51)			
Seed	Super	$Y=0{\cdot}4888+0{\cdot}1278x$ $-0{\cdot}001197x^2$	3·409(53)	3·400(50)	3·403(56)
	FYM	$Y=0{\cdot}7589+0{\cdot}0448x$	—	—	—
Fodder	Super	$Y=22{\cdot}79+10{\cdot}4168x$ $-0{\cdot}0797x^2$	340·4(65)	339·4(50)	333·3(56)
	FYM	$Y=27{\cdot}39+3{\cdot}6132x$	—	—	—
		(Average over three years)			
Seed	Super	$Y=0{\cdot}6465+0{\cdot}1015x$ $-0{\cdot}000976x^2$	2·639(52)	2·627(48)	2·626(56)
	FYM	$Y=0{\cdot}5787+0{\cdot}07927x$ $-0{\cdot}000628x^2$	2·501(63)	2·484(58)	1·962(92)
Fodder	Super	$Y=82{\cdot}61+9{\cdot}5320x$ $-0{\cdot}07144x^2$	318·0(67)	284·4(63)	309·3(56)
	FYM	$Y=91{\cdot}45+3{\cdot}7303x$	—	—	344·6(92)

Figures in brackets represent corresponding doses.

It may be noted that in all cases, except for fodder (1950-51) and for the average over three years, the maximum yield under superphosphate obtained was for values of x within the range experimented, i.e. 0 to 64 lb. The doses yielding optimum response were only a little less than those producing maximum response—the largest differences of 6 and 8 lb. being for seed for the year 1949-50 under superphosphate and farmyard manure respectively while generally the differences are from 2 to 3 lb.

Since yields of seed and fodder are related, a particular dose of manure would naturally affect the produce of both, though the extent of responses may be different. A perusal of Table IX shows that the doses of fertilizers giving responses, whether maximum or optimum, are different for seed and fodder. It is, therefore, desirable to work out one dose which would give fairly good responses in both seed and fodder. A similar dose can also be worked out for optimum responses. For this purpose it would be logical to maximise the sum of yield functions of seed and fodder. Since the yield of fodder and seed are very widely different, to maximise the sum of their yield functions without giving suitable weightage to seed would be unjustified. This procedure, however, could be applied to work out a suitable dose that would give maximum profit for seed and fodder taken together. This dose may be termed the best economic dose, hereafter referred to simply as the best dose. The profits obtained from seed and fodder with a given dose x lb. per acre are given by :

$$U_s = p_s (b_s x + c_s x^2) - qx \text{ and}$$

$$U_f = p_f (b_f x + c_f x^2) - qx \text{ respectively}$$

where U is the profit and the subscripts s and f refer to seed and fodder respectively. The best dose is obtained by maximising the function :

$$U = U_s + U_f. \text{ We get}$$

$$X_b = \frac{2q - (b_s p_s + b_f p_f)}{2 (c_s p_s + c_f p_f)},$$ where X_b is the required best dose which gives maximum profit from seed and fodder together. The values of X_b and the corresponding responses are tabulated in the last column of Table IX. Values of X_b beyond the range experimented, i.e. above 64 lb. cannot be taken seriously into account. It will be seen that the best dose of superphosphate lies between 56 and 63 lb. P_2O_5 per acre. From Figs. 2 to 9, the values corresponding to maximum (M), optimum (O) and best (B) points on the response curves have been indicated. For example in Fig. 3 a dose of 62 lb. P_2O_5 in superphosphate per acre gives the maximum response of 392·6 maunds. This is given as M (62 ; 392·6). Similar values for optimum and best doses are given as O (60 ; 392·0) and B (57 ; 389·0) respectively.

III. Relative Performance of four Classes of Fertilizers and the Interaction of Doses with classes of Fertilizers

It is possible to represent the twelve fertilizer treatments (excluding the control) in the form of a 4×3 two-way table having one factor as the quality of fertilizers (Q) with four classes F_1, S_1, F_2, S_2 and the other as the quantity or doses (D) with three classes 16 lb., 32 lb. and 64 lb. The treatment (G), i.e. superphosphate at 8 lb. P_2O_5 + farmyard manure at 8 lb. $P0_2O_5$ per acre would be represented twice. This arrangement having 10 df gives 3 df for Q, 2 df for D and the remaining 5 df for the interaction Q $\times$ D. Appendix (i) gives the 4×3 two-way tables with standard errors, CDs, etc. As regards D, a detailed study has been made in Section II which includes the control also. This arrangement is, therefore, confined to the study of Q and the interaction $Q \times D$.

Table X gives the essential features of the analysis of variance to test D and $Q \times D$. The four qualities are arranged in a descending order of the magnitudes of their means and the non-significant groups at 5 per cent are marked by a line drawn over them. The same is done for each dose where the interaction $Q \times D$ is significant.

Table X

Analysis of variance to test significance of Q, D and QD

Factor	DF	1948-49		1949-50		1950-51		Average over three years	
		Variance	F	Variance	F	Variance	F	Variance	F
				Fodder					
Q	3	6341·79	0·72	175529·60	13·19**	161318·76	19·72**	221866·39	10·53**
D	2	451882·77	5·34**	875531·63	65·76**	744143·60	90·60**	2026110·55	96·19**
Q x D	5	8385·18	0·95	11179·33	0·84	9171·43	1·12	20362·66	0·97
Error	55	8801·87		13314·30		8180·64		21063·32	
VD/VQ			71·25**		4·99		4·59		9·13
	Q	$\overline{F_2\ S_2\ F_1\ S_1}$		$\overline{S_1\ S_2\ F_2\ F_1}$		$\overline{S_2\ S_1\ F_2\ F_1}$		$\overline{S_1\ S_2\ F_2\ F_1}$	
				Seed					
Q	3	3·2535	1·25	1·5857	3·40*	5·0810	4·20*	6·0304	3·70**
D	2	22·3186	8·58**	27·3554	58·61**	38·3198	31·67**	85·5599	52·46**
Q x D	5	8·1656	3·14*	1·6802	3·60**	0·7471	0·62	3·5250	2·16
Error	55	2·5998		0·4667		1·2099		1·6309	
Vd/Vq			6·86		17·25*		7·54		14·19**
	Q	$\overline{S_2\ F_2\ S_1\ F_1}$		$\overline{S_1\ S_2\ F_2}\ F_1$		$\overline{S_2\ S_1\ F_2}\ F_1$		$\overline{S_2\ F_2\ S_1}\ F_1$	
	16	$\overline{F_2\ S_2\ S_1\ F_1}$		$S_1\ (F_1\ F_2\ S_2)$					
QD	32	$\overline{S_1\ F_1\ S_2\ F_2}$		$\overline{S_2\ S_1\ F_1\ F_2}$					
	64	$\overline{F_2\ S_2\ F_1\ S_1}$		$\overline{F_2\ S_2\ S_1\ F_1}$					

Table X reveals that:

(i) The interaction Q×D is not significant for fodder in any year and also for the average over the three years; it shows significance for 1948-49 and 1949-50 in seed. (ii) The differences between the qualities (Q) are highly significant for fodder in 1949-50, 1950-51 and average over the three years and significant for seed over the same set of years.

This shows that, for fodder, the effect of the different classes of fertilizers is independent of the doses while for seed the different doses affect the relative performance of the four types of fertilizers in 1948-49 and 1949-50. In 1948-49 the treatment 8 lb. P_2O_5 + 8 lb. farmyard manure has given significantly greater yield of seed than that due to 16 lb. superphosphate or the same quantity of farmyard manure, while for doses 32 lb. and 64 lb. the forms do not show significant differences. In 1949-50, 16 lb. superphosphate has given significantly higher yield of seed over the other forms at the same level; at 32 lb. the different classes do not differ significantly while at 64 lb. all other forms have given significantly higher yields of seed over farmyard manure. From the study of Q, it appears that, on the whole, superphosphate treatments applied singly or in combination with 8 lb. farmyard manure have done better than farmyard manure treatments applied singly or in combination with 8 lb. superphosphate.

The variances due to different doses are higher than those due to different classes—being significant for fodder 1948-49, seed 1949-50 and seed average over three years. This indicates that differences due to quantities are more pronounced than those due to qualities of fertilizers.

Discussion

A high degree of relationship was observed between the yield of green fodder and seed, both when the treatment effect was present as well as after eliminating the effect due to treatments and blocks. This implies that a fertilizer treatment that enhances the yield of berseem green fodder also enhances the yield of berseem seed.

It is, therefore, expected that the nature of responses in the two characters would be similar.

Response to superphosphate is generally quadratic while for farmyard manure the response is linear in all cases for fodder and in most cases for seed. The same is true with regard to farmyard manure at 24 lb. and 56 lb. P_2O_5 in combination with superphosphate at 8 lb. P_2O_5 per acre. For same doses of superphosphate in combination with farmyard manure supplying 8 lb. P_2O_5 per acre, the evidence is not conclusive regarding the linearity or otherwise of the response. This may be due to the presence of 11 lb. of nitrogen and 17 lb. of potash in farmyard manure and comparatively lower content of P_2O_5. It, therefore, appears that the presence of nitrogen and possibly potash in farmyard manure is largely responsible for the linear nature of response within the range of P_2O_5 examined and the quadratic nature of response in case of P_2O_5 in superphosphate may be attributed to the absence of nitrogen and possibly potash. It will be observed that, generally, larger doses of superphosphate are required to give maximum production of fodder than for seed.

The same is true with regard to doses required for optimum yields. But one would be most interested in getting maximum profits both from seed as well as from fodder for which the best dose has been found to be between 56 lb. and 64 lb. P_2O_5 per acre.

Although the responses in fodder and seed are similar, the differences between different fertilizer classes are not so for the the same dose.

For the same amount of P_2O_5 applied as superphosphate alone or mixed with farmyard manure (8 lb. P_2O_5) it has, in general, given a higher yield of berseem fodder than farmyard manure alone or mixed with superphosphate (8 lb. P_2O_5). In the case of seed the position is similar, except that the differences are less marked. As is expected, the doses have a greater effect than the qualities of fertilizers. The absence of interaction between doses and classes of fertilizers for fodder and its presence for two years for seed shows that the relative performances of different classes of fertilizers are consistant for the doses with regard to fodder, but erratic with regard to seed.

Summary

Yield data (fodder and seed) from a manurial experiment on berseem conducted during 1948-49 to 1950-51 at the Indian Agricultural Research Institute have been statistically examined with a view to studying—

(i) relationship between fodder and seed yields,

(ii) nature of response in the two characters and

(iii) the relative performance of the four fertilizer classes supplying the same doses of phosphate (16, 32, and 64 lb. P_2O_5 per acre) in the form of superphosphate, farmyard manure and combinations of the two and their interaction with the different doses.

The results of the investigation are given below:

(1) The yield of berseem fodder and that of the seed are significantly related for the three years and also for the average over three years. The correlations are highly significant for the treatment means (T) and significant after eliminating the effects due to blocks and treatments (E). The same is true with regard to linear regression coefficients.

(2) The linear equations for the treatment means and those for the error are

Years	Between treatment means	Eliminating treatments and blocks, i.e. error
1948-49	$Y=0{\cdot}9713+0{\cdot}010138x$	$Y=3{\cdot}3533+0{\cdot}005356x$
1949-50	$Y=-0{\cdot}3734+0{\cdot}004941x$	$Y=1{\cdot}1926+0{\cdot}002202x$
1950-51	$Y=1{\cdot}6219+0{\cdot}008349x$	$Y=3{\cdot}3724+0{\cdot}004062x$
Average	$Y=0{\cdot}7558+0{\cdot}007602x$	$Y=2{\cdot}8663+0{\cdot}003277x$

(3) Between themselves the correlations do not differ significantly for the three years either for E or for T. This shows that the degree of relationship between the yields of fodder and seed does not materially change with time.

(4) While the regression coefficients for E do not show any significant difference between the years, these differences for T are highly significant. In the latter case the regression coefficients for the first and third years are significantly greater, nearly twice, than the second, but do not differ significantly among themselves. This shows that the average increase in yield of seed for the first and third years, was nearly twice that over the second year for a fixed increase of yield of green fodder, the increase being highly significant when the treatment means are considered.

(5) The average increase in weight of seed for unit increase in weight of fodder is nearly twice for treatment means of that obtained after removing block and treatment effects. The differences are highly significant for the second two years.

(6) Although fodder yields due to the same amount of P_2O_5 are higher in the case of superphosphate than those due to farmyard manure, the responses are generally quadratic and linear respectively. In the case of seed, the position is similar as regards responses, but the differences in yield for the fertilizer classes supplying the same amount of P_2O_5 are less marked.

(7) The effects of the different forms of fertilizers upon the yield of fodder are independent of those due to the doses, but for seed the two factors interact for the first two years but not for the third year and also for the average over the three years.

(8) Differences in yields due to doses are more pronounced than those due to qualities of fertilizers.

(9) It is observed that at least an application of P_2O_5 varying from 56 lb. to 64 lb. per acre in the form of superphosphate is required to derive maximum profit with regard to berseem green fodder and seed production.

Acknowledgment

The authors are thankful to Dr T. J. Mirchandani, former Head of the Division of Agronomy for facilities provided and to Dr P. N. Saxena, Assistant Statistician for helpful suggestions.

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APPENDIX I

Yield of berseem fodder and seed in maunds per acre

Classes	Fodder			Mean	Classes	Seed			Mean
	Level of fertilizer (lb.)					Level of fertilizer (lb.)			
	16	32	64			16	32	64	
	L=127·72		1948-49			L=1·38		1948-49	
F_1	196·53	298·39	348·62	281·18	F_1	2·33	3·78	4·15	3·42
S_1	211·00	271·69	348·25	276·98	S_1	2·40	3·90	3·69	3·33
F_2	224·26	272·62	405·76	300·88	F_2	3·71	2·92	4·34	3·66
S_2	224·26	276·92	366·36	289·18	S_2	3·71	3·57	4·29	3·86
SE(Q.D)		±21·46	SE(Q)	±12·39	SE(Q.D)		±0·37	SE(Q)	±0·21
			C.D. at 5%	{35·20 / 43·13	C.D. at 5%		1·05	C.D. at 5%	{0·61 / 0·74
	L=94·86		1949-50			L=0·11		1949-50	
F_1	153·30	267·40	349·74	256·81	F_1	0·56	1·45	1·62	1·21
S_1	277·01	392·32	489·70	386·34	S_1	1·33	1·51	1·97	1·60
F_2	227·34	301·10	451·04	326·49	F_2	0·56	1·42	2·10	1·36
S_2	227·34	389·42	449·36	355·38	S_2	0·56	1·85	1·98	1·47
SE(Q.D)		±26·39	SE(Q)	±15·24	SE(Q.D)	±0·16		SE(Q)	±0·09
			C.D. at 5%	{43·32 / 53·03	C.D. at 5%		0·44	C.D. at 5%	{0·26 / 0·31
	L=21·38		1950-51			L=0·40		1950-51	
F_1	75·53	169·55	247·79	164·29	F_1	1·76	2·48	3·41	2·55
S_1	172·82	271·69	363·47	269·32	S_1	2·47	3·17	3·79	3·14
F_2	159·75	218·01	356·18	244·65	F_2	2·50	2·80	3·94	3·08
S_2	159·75	300·45	387·93	282·71	S_2	2·50	3·35	3·74	3·20
SE(Q.D)		±20·68	SE(Q)	±11·94	SE(Q.D)		±0·25	SE(Q)	±0·15
			C.D. at 5%	{33·95 / 41·59				C.D. at 5%	{0·41 / 0·51
	L=81·32	Average over three years			L=0·63	Average over three years			
F_1	141·79	245·11	315·38	234·10	F_1	1·55	2·57	3·06	2·40
S_1	220·28	311·90	400·47	310·88	S_1	2·07	2·86	3·15	2·69
F_2	203·78	263·91	404·33	290·67	F_2	2·26	2·38	3·46	2·70
S_2	203·78	322·26	401·22	309·09	S_2	2·26	2·93	3·84	2·84
SE(Q.D)		±19·16	SE(Q)	±11·06	SE(Q.D)		±0·17	SE(Q)	±0·10
			C.D. at 5%	{31·45 / 38·51				C.D. at 5%	{0·28 / 0·34

Note.—(i) SE(Q.D) refers to standard error of mean in the body of the table.
(ii) SE(Q) refers to standard error of mean of any one of the four classes of fertilizers taken over the doses.
(iii) The second CD at 5% below SE(Q) is the critical difference at 5% level for the comparison F_2-S_2 while the first CD is for all other comparisons.
(iv) The mean for control is given along the symbol "L".

APPENDIX II

Analysis of variance to test significance of treatment differences

	Source	Fodder			Seed	
		DF	Variance		Variance	F
1948-49	Treatments	11	123625·20	14·05†	16·1695	6·22†
	Error	55	8801·87		2·5998	
1949-50	Treatments	11	287965·91	21·63†	7·8146	16·74†
	Error	55	13314·30		0·4667	
1950-51	Treatments	11	252141·86	30·82†	19·3421	15·99†
	Error	55	8180·64		1·2099	
Average over three years	Treatments	11	615076·12	29·20†	38·2670	23·46†
	Error(1)	55	21063·32		1·6309	
	Years	2	481363·34	104·37†	244·5100	184·35†
	Years x Treatments	22	24328·42	5·27†	2·5296	1·91*
	Error(2)	110	4616·74		1·3228	

* Indicates significance at 5 per cent.
† Indicates significance at 1 per cent.

APPENDIX III

Table of Orthogonal Polynomials for n=4 when x=0, 1, 2, 4

x	Z'_1	Z'_2	Z'_3	
0	−7	+7	−3	The equation is
1	−3	−4	+8	$y=b_0+b_1Z_1+b_2Z_2+b_3Z_3$.....
2	+1	−8	−6	where $Z_0=1$
4	+9	+5	+1	$Z_1=x-\frac{7}{4}$
$\sum Z'^2$	140	154	110	$Z_2=x^2-\frac{29}{7}x+2$
L	4	7/2	55/12	$Z_3=x^3-\frac{315}{55}x^2+\frac{392}{55}x-\frac{36}{55}$
				$Z=\frac{Z'}{L}$
				$b_s=\frac{\sum yz_s}{\sum z_s^2}$

CONTAMINATION OF STORED FOOD GRAINS WITH INSECTICIDES I

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FOR the protection of grains stored in jute bags from insects, Sontakay [1950] recommended the use of BHC and DDT dusts on the surface of the bags. Surface dusting with these insecticides has, since then, been widely followed in storing cereals in this country. Little is, however, known about the contamination that such treatments would leave in the treated grains. The matter of contamination, at the same time, is of paramount importance since, no matter how effective any treatment is in preventing infestation, it can scarcely be regarded as practical if it was to be carried out at the cost of contaminating the food product. Doubts are, therefore, being expressed about large scale application of this method and the acceptibility of grain so treated for consumption.

Butterfield, Parkin and Gale [1949] mentioned the contamination passed on to the grain by DDT impregnated bags. Pingale showed the extent of contaminations with lindane impregnated bags. Similar data for bags which receive surface dusting, however, do not appear to be on record. Experiments to determine the amount of BHC passed on to the grain when used as surface dust were, therefore, carried out.

Methods and materials

The contaminations were determined in the grain, treated under laboratory and commercial storage conditions and in the grain available to the consumers from the treated lots. The insecticide used in the studies was a formulation of technical BHC containing $0{\cdot}5 \pm 0{\cdot}03$ per cent gamma-BHC.

In the laboratory tests, $1' \times 1'$ B-twill jute bags were filled with 5 lb. of Manitoba wheat each, and the insecticide was deposited on one of their outside surfaces under a settling tower. It was ascertained that by this method the insecticide settled on the bags gradually and evenly. The dusted bags were kept in a room in single layer and were not disturbed during storage. The insecticide was deposited on the bags once a month with as little disturbance to the contents as possible. The temperatures and humidities of the storage room varied between 70-84°F and 60-65 per cent respectively.

Grain samples for estimation from these bags were drawn at the end of 3, 6, 9 and 12 months' storage period and each time 100 gm. of grain was drawn from a bag. For drawing samples at the end of 3, 6 and 9 months, one side of the bag was opened and a hollow tube (6 in. long) was probed in different layers. The tube could take out about 20 gm. of grain each time. At the end of 12 months, the contents of the bag were poured out, mixed and the sample drawn from the mixture.

In the warehouse, Manitoba wheat was filled in 'B-twill' jute bags of standard size and these were piled in rectangular stacks of 1,700-2,000 bags, each (vertical layers-15). Insecticide was applied to the outside surfaces of the stacks through a hand driven rotary dusting machine at a rate of 9 cz./100 sq. ft. area of the stack. To one set of stacks, application was made at 15 days interval and to the other at 30 days interval. The dusted bags were brushed once a month. Samples from the stacks were drawn with the help of a steel 'probe'. The surface layer which received dusting and the next two layers from the inside which did not receive dusting were sampled. The 'probe' was inserted to a depth of 6 in. in each bag and about 20 gm. grain was collected. By sampling different bags about 2 lb. of grain was taken from each layer of a stack.

The sampled grain was put in air-tight containers and sent for estimation. The time between sampling and estimations varied from 6 to 8 days.

The grain from the treated lots available to the consumers was sampled at the selling points. The samples were collected from 25 and 6 different shops in two towns and from each shop about $\frac{1}{2}$ lb. of grain was taken. Similar to the grains sampled from the warehouse, this grain was put in air-tight tins and taken for estimation 6 to 8 days after sampling.

To determine the BHC content, each sample was extracted in a soxhlet with ether and subjected to dehydrochlorination by monoethanolamine [Howard, 1948]. The chlorine content was then estimated by Volhard's method and the amount of BHC calculated from it. With every set per estimation, grains that had not received any insecticidal treatment was also subjected to analysis. The test with the untreated grain was considered the blank test and the values for BHC shown have been corrected for the blank values. The sensitivity of the method employed for estimation was observed to be 0·2 p.p.m. of BHC.

Results

Laboratory tests

In the laboratory tests, insecticide was applied to the bags without any pressure at the rate of 6, 8 and 10 oz./100 sq. ft. Further, prior to a fresh application every month, the old residue was not removed from some bags and was removed with a brush from other bags. Each treatment was replicated four times. From the results shown in Table I, it is clear that where the residue was not removed, contamination to the grain did not occur but where the bags were brushed, grains got the contamination. This was probably due to the insecticide being pushed in the bags, by the brushing operation. The steady rise in the quantity of BHC in the latter case, indicates that the insecticide accumulated in the grain.

TABLE I

BHC contamination in wheat stored in jute bags which received surface dusting under a settling tower

Treatment	Concentration of BHC formulation used Oz./100 sq. ft.	Mean BHC p.p.m. found on the grain at the end of			
		3 months	6 months	9 months	12 months
Bags not brushed	6	*nil*	*nil*	*nil*	*nil*
	8	*nil*	*nil*	*nil*	*nil*
	10	*nil*	*nil*	*nil*	*nil*
Bags brushed	6	0·7	2·2	4·0	6·0
	8	0·5	1·5	3·5	5·0
	10	1·0	2·5	4·5	6·5

The contamination occurring under warehouse conditions for a storage period of 6 months is shown in Table II. In this case, the insecticide showed a tendency to accumulate in the surface layers and this was seen to increase with the number of dustings. One sample, drawn from the third layer of the stack also showed the presence of the insecticide in the grain. In the usual process this layer had little chance of getting an insecticide on it. The contamination observed has, therefore, probably occurred in the process of sampling.

TABLE II

BHC contamination in the grains, stored and treated under commercial warehouse conditions

Treatment	Sample drawn from	Mean BHC p.p.m. found in the grain at the end of	
		3 months	6 months
Dusted every 15 days	Surface layer	3·2	5·4
	2nd layer	..	..
	3rd layer	..	..
Dusted every month	Surface layer	2·2	3·6
	2nd layer	..	..
	3rd layer	..	14·0

The contamination observed in the marketed grains is shown in Table III. In this the first 25 samples were drawn from one town and the rest from another town. In relation to the previous results, the contaminations here were uneven. This was expected since only the surface layers of the grain stacks received dusting. The very high BHC content shown in 2 samples in Town-1 and one in Town-2 could have been due to the mixture of grain sweepings with the grain in bags. In storage, some grains spill out from bags and this gets heavily contaminated with BHC which is present on the warehouse floor. The spilled grains are usually accumulated and to avoid losses are mixed with the grain in bags. The particular lot of bagged grain then is likely to carry a heavy contamination of the insecticide.

A few samples of spilled grain collected from the floor of a treated warehouse showed the presence of 647-1423 p.p.m. BHC on them after mechanical cleaning. This suggests the extent to which the grain collected from the treated floors would contaminate the other grains, with which they might be mixed.

Table III

BHC contamination in the grains available to the consumers

Sample No.	BHC contamination p.p.m.	Sample No.	BHC contamination p.p.m.	Sample No	BHC contamination p.p.m.
1	*nil*	11	1·0	21	1·7
2	*nil*	12	1·0	22	*nil*
3	*nil*	13	*nil*	23	*nil*
4	0·6	14	*nil*	24	*nil*
5	0·8	15	*nil*	25	*nil*
6	*nil*	16	*nil*	26	1·5
7	1·6	17	69·6*	27	*nil*
8	*nil*	18	*nil*	28	*nil*
9	*nil*	19	12·5	29	62·0*
10	47·5	20	*nil*	30	*nil*

* These grains had a normal flavour but a slight bitter taste.

Discussion

The use of an insecticide in the protection of a food material involves two aspects namely, the extent of protection it offers and the toxicity it leaves behind through the residues to the consumers of the treated food. In this study which is confined only to the latter aspect the results suggest that when BHC is used for surface dusting at the rate recommended by Sontakey [1950] the residue passed on to the grain is not appreciable. The World Health Organisation has suggested the permissible limit for BHC as 5 p.p.m. [Hanna 1952]. In the light of this suggestion the residues observed in the wheat from treated bags cannot be considered injurious to the consumers. The practice of mixing spilled grain with the grain in bags is, however, suspected to introduce heavy insecticide contaminations in the grain. Sontakey [1950] recommended washing of the grain spilled on the insecticide treated floor with water, prior to its mixing with the grain in bags. From the results it appears that either such a washing does not remove all the insecticide or the method is not scrupulously followed. Further, in normal practice it would be extremely difficult to see that washing is done and the chances are that the individuals would mix every grain fallen on the treated floor with the grain in the bags without washing. The risk to the consumers in such cases will be greater, particularly when cereal grains form the main item of diet of the people.

In the light of existing knowledge, there will be no two opinions in suggesting use of enlightened caution with regard to the insecticide residues on foods. The advantages of using the pesticide as represented by improvement in the nutrition of a population must be set against the hypothetical risks incurred. It is also well to consider whether residue hazards cannot be reduced without loosing the desired effect.

Lehman [1954] considers the use of lindane (gamma—BHC) reasonably safe because of its minimum body storage and rapid elimination from the body tissues. Being very active, for similar effect lindane is required in about 1/10 the quantity of BHC and the permissible limit for its presence in the food is the same as that of BHC [Hanna 1952]. Its use for surface dusting in place of BHC is, therefore, likely to reduce the toxicity hazard to the consumer.

Pingale showed that very small quantities of lindane when impregnated on jute bags protected the grains from insects efficiently. Such impregnations further, appear not to leave injurious contaminations on the grains stored in the bags. The impregnated bags would not also contaminate the spilled grain and the method of impregnation at the same time being simple and cheap, could be followed to avoid contaminations.

Butterfield, Parkin and Gale [1949] reported that the grain, from bags subjected to handling got a relatively large contamination of the insecticides. Since, the grain bags which receive surface dusting are frequently moved and even trampled over, contaminations under these conditions are being studied and will form the subject of another paper.

Summary

Jute bags containing wheat were subjected to surface dusting with BHC under laboratory and commercial storage conditions. The extent of contamination introduced by the dusting in the grain is shown which suggests that mere surface dusting does not leave large residues of the insecticide in food. In the further examination of grain from treated bags available to the consumers, however, some lots were observed to contain large quantities of the insecticide. This presence of unusually large quantity was considered due to the practice of mixing spilled grain with the grain in bags. In view of the findings it was concluded that either an insecticide of much higher potency against insects but with a lower or similar toxicity to higher animals might be used in place of BHC or a chemical test to detect the insecticide residue in the spilled grains be employed prior to their mixing with the grain in bags.

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PLANTS SUITABLE FOR SOIL CONSERVATION

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THE Soil is most susceptible to erosion when the fields are bare of vegetation. Evidences support that the soils low in organic matter content or in fertility and poor in soil structure are more easily eroded than those high in organic matter, humus and water stable aggregates.

When grasses and legumes secure a foothold, the soil materials are held in place by the network of root system. Old roots die and new ones take their place. A part of the root material and a part of the annual crop of grass and legume tops added, remain to form the organic matter (humus) in the soil, improving the organic matter status and physical condition of the soil and ultimately the soil fertility. Thus the soil covered with grasses and legumes, containing living and dead plant parts, living and dead organisms and humus, bound with the extensive root system of grasses, and provided with thick vegetative cover is protected from erosion. Because of these qualities, grasses and legumes have proved to be the best agents in controlling the soil erosion, specially under such conditions where nothing else can grow.

During the intensive studies at Indian Agricultural Research Institute, New Delhi, on the role of the grasses and legumes, the plant material discussed below has been found useful in successfully protecting the soil from erosion, particularly in regions where soil and climatic conditions are not favourable for the establishment of forest.

Grasses

There are a number of grasses that can be introduced for controlling soil erosion but only important twelve dual purpose grasses (for fodder and erosion control) are briefly described.

Cynodon plectostachyum (Giant star grass). This perennial grass has been introduced in India from Africa. The grass sends out long runners, which strike roots at nodes. The shoots grow fast during spring and monsoons and cover up the soil quickly and thoroughly. The network of runners forms 12in.-18in. thick mat. The grass is easily propagated by root-cuttings during monsoon. No seed setting has been observed under Delhi conditions. The grass is winter killed and fairly drought resistant. It is being tried by Damodar Valley Corporation for soil conservation.

2. *Panicum repens* (Torpedo grass). This indigenous grass was originally collected from Coonoor. This perennial grass is specially suited for sandy soils where it makes a rapid growth. The grass is very aggressive and quickly spreads to adjoining areas. The growth is very vigorous during spring and summer.

The grass gets propagated by the underground runners; it can also be established by seed but is established more quickly by rooted runners during monsoons. The underground runners give out new shoots like a torpedo and are, therefore, known as the Torpedo grass. Once established, the grass is difficult to be eradicated by ordinary methods.

3. *Pennisetum clandestinum* (Kikuyu grass). This perennial grass grows during monsoon and early winter. The grass is propagated by rooted slips or underground runners. No seed setting has been observed under Delhi conditions. The grass shows quick growth on soils of medium fertility in high rainfall tract. The underground runners and thick vegetative growth bind and cover the soil, protecting it from erosion. Well established grass is difficult to be eradicated. It is not suitable for dry areas.

4. *Urochloa species*. *Urochloa* species and *Urochloa mosambicensis*, the two species of *Urochloa* have been introduced from Australia. These grasses are perennial and have prostrate growth. The branches strike roots freely at nodes, covering the soil quickly and thoroughly. The seed setting is excellent in both the species. The grasses can be established both by seed and rooted slips and show rapid growth during spring and monsoon. Both the species are partially resistant to low temperatures during winter and fairly resistant to drought. These grasses, due to their free rooting habit and capacity to produce thick vegetative cover, are well suited for soil conservation.

5. *Brachiaria mutica* (Para grass). This perennial grass is indigenous to India and remains green throughout the year under irrigated conditions. It sends out long, soft and hollow stems freely striking roots at every node. The network of stems forms a thick mat, rising to 4-5 feet above the ground. The grass quickly recovers after cutting and provides 7-8 cutting of green fodder per year. No seeding has been observed under Delhi conditions. It is easily established by rooted cuttings or rooted slips during spring and monsoon. The grass is well suited for high rainfall tracts and can very well stand water-logged or alkaline soils. It cannot survive in droughty conditions.

6. *Brachiaria brizantha*. This grass has been introduced from Australia. It is a perennial grass and remains green throughout the year under irrigated conditions. The grass compares well with drought and cold resistant grasses. It rapidly covers the soil in early spring and summer with its vegetative shoots running along the ground. The grass is very leafy and forms a thick cover. The seed setting is profuse. It can be propagated both by seed and rooted slips.

The other two species, *Brachiaria lata* and *Br. intermedia*, seem to be equally promising; these are leafy, quick growing and strike roots at nodes.

7. *Bothriochloa insculpta*. This perennial grass is an introduction from the U. S. A. It spreads sending out runners all around; the runners strike roots at nodes. The shoots show quick growth and thick cover. The network of runners

forms a thick mat covering the soil perfectly. The grass is not well suited for droughty conditions but it is winter hardy. The seed setting is poor and the grass can be propagated only by root cuttings or slips.

8. *Eragrostis lehmaniana* (Love grass). The genus Love grass, *Eragrostis* comprises many species. Few species have an agricultural value. *Eragrostis lehmaniana* is recognized for its capacity to produce abundant seeds and forages on the soils of medium to low fertility. The grass resists summer heat and drought and survives frost, if adequate moisture is present in it. The Love grass is easily established from seed and volunteers aggressively and its vigorous young seedlings quickly make an effective green cover.

9. *Paspalum dilatatum* (Dallis grass). Paspalum grasses are primarily pasture grasses. Some are short-lived but most of them maintain good stand and remain productive for long time if properly managed and fertilized. The grass is winter hardy but cannot stand drought. The nodes strike roots wherever they touch the moist soil. The grass produces abundant seeds. The rooting habit and thick vegetative cover combined with winter hardiness makes the grass suitable for controlling soil erosion. The grass can be propagated both by seed and rooted slips.

10. *Chloris gayana* (Rhodes grass). This grass is fine stemmed and very leafy ; grows approximately three feet high. The plants also spread by running branches and stolens that are 3-5 feet long and root and produce a plant at every node. The grass is winter hardy if the soil contains an adequate amount of moisture, and can stand droughty conditions and grows during several months of drought. The seed production capacity is excellent. The grass can be established from seed and planting material and grows aggressively. Its vigorous growth during monsoons produces an effective green cover.

11. *Dichanthium annulatum*. This grass is fairly well known throughout the country under different names. It is common Apang grass of Delhi State. The grass is suitable for both light and heavy soils. The collection from Karnal shows vigorous prostrate growth during monsoons and covers the soil thoroughly with its vegetative growth, quickly. The grass can be established both by seed and planting, during rainy season. It shows early spring growth but does not contain sufficient leafy matter. The grass resists drought and responds well to irrigation.

12. *Digiteria species* (Crab grass). The genera consists of many grasses. The indigenous species collected from Bhowali showed promise for controlling soil erosion. The grass showed prostrate growth, the branches running along the ground root freely and forming independent plants at nodes. The plants are very leafy and quick growing, covering the soil in short period of about 8 weeks after transplanting the seedlings. It has good capacity to set seed. The grass can be established both by seed and planting material.

Legumes

Amongst legumes, there are few important, which can be profitably utilized both for fodder and soil conservation and have been briefly described here.

1. *Pueraria hirsuta* (Kudzu vine). It is a perennial legume widely accepted for soil conservation in the U. S. A. Except waterlogged and alkaline soil, it can grow on all soils. Kudzu is an ideal plant for rapid control of soil erosion for light well drained poor soil commonly occurring in northern India.

The plant spreads vines in all directions, and under favourable conditions the length of vine extends 50 feet and above. The vines strike roots at nodes, and each node behaves as an individual plant in the next season and further sends out new shoots which cover the soil rapidly. The shoots of well developed plants showed rapid growth of 8·7 cm. per day during monsoon. The plant dries up completely during winter. The new growth commences during early spring and the plant remains green during hot summer and autumn months. The well established plants do not require irrigation. A plant introduced at I. A. R. I. during 1945 has never been irrigated except for the first two years and it now presents an excellent stand of 4-5 feet height and cover an area of 0·14 acre. Kudzu is best propagated by crowns, which are available during December-January when the vine is dormant.

2. *Dolichos lablab* var. *lignosus*. This is a creeping perennial legume widely growing round about Coimbatore. The long inter-twinning, wiry vegetative shoots form a thick mat and an excellent cover. Since the rooting habit of the plant is poor, the mat can be rolled and unrolled. The pod formation and seed setting is excellent and large quantities of seeds can be collected, but the pods are damaged by frost under Delhi conditions, resulting in low yield of seed. The plants are easily established by seed and under favourable conditions provide excellent cover during first season of its growth. The plants are severely damaged by frost. The mat, however, remains intact protecting the soil from wind erosion. The fresh growth appears during spring but the active growth commences 2-3 weeks before the commencement of monsoon, providing thick cover for the eroding soil.

3. *Glycine javanica*. This is also a creeping perennial legume and is closely related to soybean. The vine strikes roots forming an independent plant at every node. The rooting habit and thick leafy cover bind and protect the soil against erosion. Seed production is very poor under Delhi conditions due to low temperatures at the time of flowering and pod formation. Good quantity of seed can be harvested under milder climatic conditions. The plants dry up during winter and commence active growth late in spring and provide a thick vegetative cover before monsoon.

4. *Centrosema pubescens*. The twinning, herbaceous, perennial legume freely roots at nodes, providing a dense green carpet during late spring, monsoon and autumn. The legume is winter affected and commences new growth late in spring. The seed production is poor under Delhi conditions as the pods are frequently affected by frost. It is comparatively slow growing legume and cannot grow under drought conditions but requires a long wet season for quick cover.

5. *Indigofera endecaphylla*. Indigoferas are well adapted to poor soils and are generally first to invade the barren areas. The stemmy growth of the legume

provides a few-inch thick carpet for soil during monsoons, which proves an excellent cover in protecting the soil from erosion. It is frosted in winter and remains dormant in dry season. It requires long wet season for quick and aggressive growth. The legume can be established both by seed and rooted cuttings during monsoons. The seed setting is profuse.

6. *Desmodium scorpiurus*. This legume is a recent introduction from Australia. It is a creeping perennial legume, freely rooting at nodes, providing a dense few-inch thick cover throughout the year. The legume has resisted frost and shown active growth during spring and monsoons. The legume is propagated by seeds which are formed in plenty. It has proved promising for checking soil erosion and is under further observation.

Annual leguminous cover crops. Cowpeas (*Vigna sinensis*), Velvet bean (*Mucuna cochinchinensis*), are two important legumes, which when sown at the commencement of monsoon quickly cover the soil. The cowpeas provide cover till the end of October but the velvet beans remain green till the end of November and are ready for harvest in December.

Vicia sativa, a common vetch, affords protection to soil from December to May while Bur clover (*Medicago hispida*) provides a cover from January to March. Both of these are known for their soil improving, green, manuring, fodder value and affording an excellent soil cover. They also possess a desirable quality of regeneration every year without reseeding.

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